การประเมินผลการตรวจกรองธาลัสซีเมียและฮีโมโกลบิน อี ในโรงพยาบาลชุมชน

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ศูนย์อนามัยที่ 9 นครราชสีมา 177 หมู่ 6 ตำบลโคกกรวด อำเภอเมือง จังหวัดนครราชสีมา

บทคัดย่อ

งานวิจัยนี้เป็นการศึกษาเชิงพรรณนาแบบศึกษาข้อมูลย้อนหลัง มีวัตถุประสงค์เพื่อประเมินการใช้เกณฑ์อ้างอิงของประเทศในการตรวจกรองธาลัสซีเมียและฮีโมโกลบิน อี ในหญิงตั้งครรภ์และคู่สมรสของโรงพยาบาลภาครัฐ (ค่า MCV < 80 fL และ MCH < 27 pg ร่วมกับการตรวจกรองฮีโมโกลบิน อี โดยการตกตะกอนด้วยดีซีไอพี) เปรียบเทียบกับวิธีมาตรฐาน ได้แก่ การตรวจหาชนิดและปริมาณฮีโมโกลบิน และการตรวจดีเอ็นเอ กลุ่มตัวอย่างเป็นตัวอย่างเลือดหญิงตั้งครรภ์และสามี จำนวน 7,615 ราย ที่ให้ผลบวกกับการตรวจกรองเบื้องต้น และฝากครรภ์ ณ คลินิกฝากครรภ์ โรงพยาบาลชุมชน 63 แห่ง ใน 4 จังหวัด ของภาคตะวันออกเฉียงเหนือ ได้แก่ จังหวัดนครราชสีมา ชัยภูมิ บุรีรัมย์ และสุรินทร์ โดยตรวจกรองพาหะ α^0-thalassemia และพาหะ β-thalassemia ในตัวอย่างเลือด 7,615 ตัวอย่าง ด้วยเครื่องวิเคราะห์เม็ดเลือดอัตโนมัติ 9 ยี่ห้อ และ ตรวจกรองฮีโมโกลบิน อี ในตัวอย่างเลือด 6,382 ตัวอย่าง โดยการตกตะกอนด้วยดีซีไอพี เมื่อเปรียบเทียบผลการตรวจกรองกับวิธีมาตรฐาน พบว่าเกณฑ์อ้างอิงของประเทศที่ใช้ค่า MCV < 80 fL และ MCH < 27 pg สามารถใช้ได้กับเครื่องวิเคราะห์เม็ดเลือดอัตโนมัติ ยี่ห้อ Coulter DxH500 ยี่ห้อ Coulter LH780 ยี่ห้อ Dirui BF 6800 ยี่ห้อ Pentra ES60 ยี่ห้อ Pentra XL80 ยี่ห้อ Cell-Dyn Ruby ยี่ห้อ Sysmex XN- 1000i และยี่ห้อ Sysmex XS-800i แต่มีตัวอย่างเลือดพาหะ β^+-thalassemia จำนวน 1 ตัวอย่าง ที่ตรวจวิเคราะห์ด้วยเครื่องยี่ห้อ Mindray BC6800 มีค่า MCV > 80 fL และ MCH > 27 pg อย่างไรก็ตามไม่สามารถระบุได้ชัดเจนว่าค่า MCV< 80 fL และ MCH < 27 pg ใช้กับเครื่องยี่ห้อ Mindray BC6800 ไม่ได้ เนื่องจากจำนวนตัวอย่างที่พบเพียง 1 ตัวอย่าง ไม่สามารถแสดงความน่าเชื่อถือของข้อมูลได้ ส่วนการตรวจกรอง Hb E โดยการตกตะกอนด้วยดีซีไอพี พบค่าการทำนายผลบวก ค่าการทำนายผลลบ และค่าความถูกต้อง เท่ากับร้อยละ 93.8 83.2 และ 89.5 ตามลำดับ ซึ่งปัจจัยที่มีผลต่อการแปลผลผิดพลาด คือบุคลากรห้องปฏิบัติการขาดความรู้และทักษะในการอ่านผล และห้องปฏิบัติการขาดระบบควบคุมคุณภาพที่ดีเพียงพอ ผลการศึกษาครั้งนี้สรุปได้ว่าการตรวจกรองธาลัสซีเมียโดยใช้ค่าดัชนีเม็ดเลือดแดงร่วมกับการตรวจ Hb E ในกลุ่มหญิงตั้งครรภ์และสามี ตามแนวทางการตรวจกรองธาลัสซีเมียของประเทศ ห้องปฏิบัติการแต่ละ

แห่งควรใส่ใจเกี่ยวกับผลลบลวง และควรมีระบบการควบคุมคุณภาพที่ดี นอกจากนี้บุคลากรผู้รับผิดชอบการตรวจกรองควรได้รับการอบรมอย่างสม่ำเสมอเพื่อเพิ่มขีดความสามารถในการปฏิบัติงานได้อย่างถูกต้อง

คำสำคัญ: การตรวจกรองธาลัสซีเมียและฮีโมโกลบิน อี เครื่องวิเคราะห์เม็ดเลือดอัตโนมัติ เกณฑ์ค่าอ้างอิง

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Evaluation of the Thalassemia and Hemoglobin E Screening in Community Hospitals

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Abstract

The purpose of this retrospective descriptive study was to evaluate the use of mean corpuscular volume (MCV) values less than 80 fL and mean corpuscular hemoglobin (MCH) values less than 27 pg combined with the dichlorophenolindophenol test (DCIP test) in a national screening protocol for thalassemia and hemoglobin E (Hb E) screening of pregnant women and their partners in community hospitals. The interpreted results were compared with the results obtained from Hb typing and DNA analysis for α^0-thalassemia and β-thalassemia as gold standard. Study participants consisted of 7,615 Thai pregnant women and their partners who were positive on the preliminary screening and attending antenatal care services in 63 community hospitals of the 4 provinces; Nakhon Ratchasima, Chaiyaphum, Buriram, and Surin. The 7,615 blood samples were initially screened for α^0-thalassemia and β-thalassemia by using 9 different hematology analyzers. A total of 6,382 blood samples were tested for the presence of Hb E by using DCIP kit. The screening results were compared with the results of Hb typing and DNA analysis. The study reveals that the Coulter DxH500, Coulter LH780, Dirui BF 6800, Pentra ES60, Pentra XL80, Cell-Dyn Ruby, Sysmex XN-1000i, and Sysmex XS-800i could be used to detect α^0-thalassemia and β-thalassemia with the reference cutoff values of MCV < 80 fL and MCH < 27 pg, but only one sample of β^+-thalassemia with MCV > 80 fL and MCH > 27 pg was detected by Mindray BC6800. However, this did not indicate that the cutoff values of MCV< 80 fL and MCH < 27 pg were not suitable for Mindray BC6800 as only one sample might not represent significant data. Hb E screening with DCIP test revealed 93.8% positive predictive value, 83.2% negative predictive value and 89.5% accuracy. It was also found that the factors affecting the misinterpretation were the lacking of knowledge and interpretation skill of laboratory personnel and insufficiency of quality control systems of the laboratories. Based on these results, using the combined test of blood indices and Hb E screening for pregnant women and their partners following the national screening protocol, all clinical laboratories

should be concerned over false negative results and the proper quality control system. Moreover, personnel who are responsible for the screening should get a competent training.

Keywords: Thalassemia and Hb E screening, Hematology analyzers, Reference cutoff values

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Introduction

Thalassemia and hemoglobinopathies are the most common inherited disorders of hemoglobin (Hb) synthesis and represent a major public health problem in Thailand. It is estimated that 30 to 40% of the population are carriers and about 1% of the population have the diseases.[1] The Ministry of Public Health established a national program for prevention and control of thalassemia in 1994. The goal of this program is to reduce the number of births with major thalassemia diseases including Hb Bart's hydrops fetalis, homozygous β-thalassemia, and β-thalassemia/Hb E. All pregnant women and their partners have the right to be screened for thalassemia without having to pay under the Total Health Care Policy. The government hospitals and private hospitals give basic services in detecting at risk couples before a child is born. Therefore, the people included in the prevention and control program are carriers of α^0-thalassemia, β-thalassemia, and Hb E.[2]

In Thailand, a national screening protocol for thalassemia and Hb E in pregnant women follows conventional guidelines. It relies on hematologic index cutoff values with mean corpuscular volume (MCV) less than 80 fL or mean corpuscular hemoglobin (MCH) less than 27 pg using an automated blood cell counter combined with the dichlorophenolindophenol (DCIP) precipitation test. Individuals with positive results should be examined further to confirm the diagnosis of α^0-thalassemia, β-thalassemia, and Hb E through Hb analysis and DNA analysis.[3, 4] However, currently clinical laboratories use various types of hematology analyzers and Hb E screening by the DCIP test. This requires highly skilled laboratory personnel. The objective of this study was to evaluate the use of MCV < 80 fL and MCH < 27 pg combined with DCIP test for thalassemia and Hb E screening of pregnant women and their partners in community hospitals compared with the gold standard.

Materials and Methods

Subjects

A total of 7,615 fresh EDTA- anticoagulated blood samples of pregnant Thai women and their partners were collected at antenatal care clinics from October 2015 to February 2017 from 63 community hospitals in the 4 provinces; Nakhon Ratchasima, Chaiyaphum, Buriram, and Surin provinces in northeast Thailand and were screened for thalassemia. We used hematology analyzers for screening of α^0-thalassemia and β-thalassemia and KKU-DCIP-Clear reagent kits for Hb E. The screening was done by technicians at the clinical laboratories in the community hospitals within 24 hours after the blood samples were drawn. All blood samples that were positive at the preliminary screening were transferred on ice to our laboratory at the Regional Health Promotion Center 9, Nakhon Ratchasima for further confirmation with Hb typing and real-time gap PCR with SYBR Green I and high resolution melting (HRM) analysis.

Hematologic Analysis

A total of 7,615 fresh EDTA-anticoagulated blood samples were determined red blood cell count indices using the 9 different hematology analyzers as summarized in Table 1.

Table 1 RBC indices of 7,615 EDTA-anticoagulated blood samples determined by using the 9 different hematology analyzers

Hematology analyzer	Total blood samples (%)	Number of blood samples of 3 thalassemia genotypes		
		Heterozygous α^0-thal	Heterozygous β-thal	Others
Coulter DxH500	302 (4.0 %)	6 (1.8 %)	5 (3.2 %)	291 (4.1%)
Coulter LH780	807 (10.6 %)	48 (14.3 %)	33 (21.0 %)	726 (10.2%)
Dirui BF 6800	425 (5.6 %)	14 (4.2 %)	6 (3.9 %)	405 (5.8%)
Mindray BC6800	1,381 (18.1 %)	67 (19.9 %)	31 (19.7 %)	1,283 (18.0%)
Pentra ES60	915 (12.0 %)	41 (12.2 %)	18 (11.5 %)	856 (12.0%)
Pentra XL80	937 (12.3 %)	55 (16.4 %)	17 (10.8 %)	865 (12.1%)
Sysmex XN1000i	453 (6.0 %)	21 (6.2 %)	9 (5.7 %)	423 (5.9%)
Sysmex XS-800i	1,807 (23.7 %)	71 (21.1 %)	26 (16.6 %)	1,710 (24.0%)
Cell-Dyn Ruby	588 (7.7%)	13 (3.9%)	12 (7.6%)	563 (7.9%)
Total	7,615 (100%)	336 (100%)	157 (100%)	7,122 (100%)

Hb E screening

A total of 6,382 fresh EDTA-anticoagulated blood samples were analyzed by using Hb E screening with KKU-DCIP-Clear reagent kits (PCL Holding, Bangkok, Thailand), following the manufacturer's protocol as described.[5]

Hb analysis

Hb patterns and levels were determined using an automated high performance liquid chromatography system (Variant, Bio-Rad Laboratories, Hercules, CA).[6] This cation-exchange column chromatography generates chromatogram and area percentage of Hb A_2, Hb E, Hb F and abnormal hemoglobins. However, Hb A_2 and Hb E were eluted by two phosphate buffers that differ in pH and ionic strength at the same window. Samples with Hb A_2 levels 3.6%-9.0% were β-thalassemia trait while the values of Hb A_2 levels higher than 9.0% were diagnosed as Hb E.

DNA analysis

Genomic DNA was prepared from peripheral blood leukocytes using QIA amp® DNA Mini Kit (QIAGEN GmbH, QIAGEN Strasse1, Hilden, Germany), according to the manufacturer's instruction. Identification of α^0-thalassemia (SEA deletion and Thai deletion) was performed using the real-time gap PCR with SYBR Green I and high resolution melting (HRM) analysis as described elsewhere.[7] Common β-thalassemia mutations in Thailand were also performed in samples with Hb A_2 level exceeding 3.5% using the real-time gap PCR with SYBR Green I and high resolution melting (HRM) analysis.[8]

Statistical Analysis

Descriptive statistics such as mean and SD were used to describe hematologic features of the subjects. To evaluate the effectiveness of Hb E screening, a positive predictive value, negative predictive value and accuracy were calculated. The results of Hb analysis and DNA analysis were used as "gold standards".

Results

The results of thalassemia genotyping and hematologic characteristics of 7,615 pregnant women and their partners are summarized in Table 2. From the 7,615 cases, 5,347 (70.2%) were found to carry thalassemia or hemoglobinopathies. In the former group, 16 thalassemia genotypes were observed. The three targets of the prevention and control thalassemia program were 336 (4.4%) of α^0-thalassemia trait, 157 (2.1%) of β-thalassemia trait, and 3,144 (41.2%) of Hb E trait. As expected, the most common genotype was Hb E, although co-inheritance with other thalassemia genotypes were also observed. The reference cutoffs of MCV < 80 fL and MCH < 27 pg for screening α^0-thalassemia were used for all of the 9 different hematology analyzers. One case of β^+-thalassemia trait (nucleotide-31 mutation) was found using Mindray BC6800 to be 81 fL for MCV and 27.5 pg for MCH and another case of β^0-thalassemia trait (codon 95) was also found using Cell-Dyn Ruby to be 83.5 fL for MCV and 24.7 pg for MCH. The data are shown in Fig. 1, and Fig. 2. For the Hb E screening with DCIP test, positive predictive value, negative predictive value and accuracy were 93.8%, 83.2%, and 89.5%, respectively (Table 3).

Discussion

Based on these results, 16 thalassemia genotypes were identified in this group of pregnant Thai women and their partners. From all blood samples that were positive at the preliminary screening, marked reduction in MCV and MCH values were observed in the group of non-clinically significant thalassemia with the mean MCV of 76.7 fL and MCH of 25.2 pg. Sanchaisuriya *et al.* showed that women with mild forms of thalassemia, α^+-thalassemia, Hb CS, and Hb Pakse could generate a value of MCV and MCH lower than

Table 2 Thalassemia genotypes and the corresponding hematologic characteristics observed in 7,615 pregnant women and their partners. Data presented as mean (SD)

Subject	N	%	RBC (x1012/L)	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	Hb Type	%A2/E	%F
Non-clinically significant thalassemia*	2,268	29.9	5.1 (0.7)	12.8 (1.8)	39.1 (5.4)	76.7 (5.2)	25.2 (2.2)	A_2A	2.9 (0.3)	0.4 (0.3)
Heterozygous α^0-thal	336	4.4	5.9 (0.7)	12.5 (1.6)	39.3 (5.2)	66.3 (3.7)	21.1 (1.2)	A_2A	2.8 (0.3)	0.4 (0.3)
Heterozygous β-thal	157	2.1	5.6 (0.7)	11.9 (1.7)	37.5 (5.3)	66.5 (5.3)	21.2 (1.9)	A_2A	5.6 (0.6)	1.4 (1.3)
Heterozygous Hb Constant Spring	384	5.0	5.0 (0.7)	12.3 (1.7)	38.1 (5.1)	75.6 (3.7)	24.4 (1.6)	CSA_2A	2.8 (0.3)	0.7 (0.5)
Heterozygous Hb E	3,144	41.2	5.0 (0.6)	12.6 (1.7)	38.2 (5.2)	76.1 (5.3)	25.2 (1.9)	EA	24.8 (5.3)	1.1 (0.8)
Heterozygous Hb E with Heterozygous α^0-thal	151	2.0	5.5 (0.7)	11.9 (1.6)	36.8 (4.9)	66.5 (3.7)	21.6 (1.1)	EA	19.6 (0.9)	0.9 (0.6)
Heterozygous Hb E with Heterozygous α^{ConSp}	88	1.1	5.0 (0.7)	11.7 (1.6)	36.6 (5.0)	73.3 (4.9)	23.5 (2.0)	CSEA	21.0 (2.3)	1.7 (1.2)
Heterozygous Hb E with α^0-thal / α^+-thal	43	0.6	6.0 (1.0)	9.9 (1.4)	31.9 (5.2)	53.6 (6.0)	16.6 (1.5)	EABart's	15.0 (1.4)	1.2 (0.9)
Heterozygous Hb E with α^0-thal / α^{ConSp}	5	0.1	5.0 (0.8)	8.2 (1.4)	28.8 (5.3)	57.2 (4.4)	16.3 (0.8)	CSEABart's	16.2 (3.7)	1.5 (0.6)
Homozygous Hb E	906	11.9	5.4 (0.8)	11.2 (1.5)	33.7 (4.9)	62.2 (4.9)	20.6 (1.3)	EE	81.1 (3.5)	3.6 (2.2)
Homozygous Hb E with α^0-thal	17	0.2	5.4 (0.6)	11.5 (1.3)	34.1 (4.0)	63.7 (6.8)	21.4 (2.0)	EE	82.2 (4.5)	2.7 (1.1)
Homozygous Hb E with Heterozygous α^{ConSp}	4	0.1	5.1 (0.8)	11.0 (1.2)	31.9 (2.8)	63.7 (6.3)	21.8 (1.2)	CSEE	81.9 (1.2)	3.4 (0.3)
Homozygous Hb E with α^0-thal / α^+-thal	1	0	5.0	8.1	25.1	50.4	16.3	EEBart's	86.3	1.6
α^0-thal / α^+-thal	64	0.8	5.4 (0.8)	9.6 (1.4)	32.5 (5.0)	60.1 (5.2)	17.9 (1.3)	A_2ABart'H	1.8 (0.4)	0.4 (0.6)
α^0-thal / α^{ConSp}	11	0.1	4.2 (0.6)	8.0 (1.4)	27.3 (5.1)	65.0 (5.9)	19.0 (1.1)	CSA_2ABart'sH	1.2 (0.5)	0.5 (0.4)
β^0-thal/HbE	20	0.3	4.4 (0.8)	9.2 (2.1)	28.5 (4.7)	63.4 (7.3)	20.9 (8.7)	EF	54.9 (13.4)	34.1 (13.6)
β^+-thal/HbE	16	0.2	5.1 (1.2)	10.1 (2.4)	31.9 (6.7)	62.8 (4.8)	20.3 (2.5)	EFA	54.4 (8.2)	14.6 (6.9)
Total	7,615	100								

* identification of α^+-thalassemia gene was not performed.

Table 3 Positive predictive value, negative predictive value and accuracy of Hb E screening using KKU-DCIP-Clear test in 6,382 pregnant women and their partners

KKU-DCIP-Clear test	Hb Typing		Total
	Hb E	Non Hb E	
Positive	3,573	237	3,810
Negative	430	2,142	2,572
Total	4,003	2,379	6,382

positive predictive value = 3,573 x 100 / 3,810 = 93.8 %
negative predictive value = 2,142 x 100 / 2,572 = 83.2 %
accuracy = 5,715 x 100 / 6,382 = 89.5 %

those in women without thalassemia.[9] However, those carriers, of which there are a high prevalence in this geographic area[10] were not identified because they are not the targets for the prevention and control of thalassemia program in Thailand. They were included in the non-clinically significant thalassemia group. Therefore, it is possible that some blood samples in this group were a mild form of α^+-thalassemia carriers that leads to the low value of MCV and MCH. As expected, Hb E was the most common hemoglobinopathy which confirms a high prevalence in Southeast Asia, especially in the northeast of Thailand.[5, 11]

The goal of screening thalassemia and Hb E for pregnant women and their partners in Thailand is to offer carrier testing in detecting at risk couples before a child is born. The screening of α^0-thalassemia, β-thalassemia are based on cutoff values for MCV and MCH by hematology analyzer and Hb E screening by DCIP test. Couples with low MCV (< 80 fL) and low MCH (< 27 pg) or positive DCIP test have further investigation using electrophoresis or HPLC or DNA analysis. Therefore, false negative results of screening should be a concern, because if a pregnant woman is found to be non-thalassemia or non-clinically significant thalassemia, it is not necessary to call for screening in her partner. Due to the fact that "silent" β-thalassemia trait with normal MCV (> 80 fL) has been reported in other countries[12] and currently clinical laboratories use various types of hematology analyzers, appropriate cutoff values of MCV and MCH of each analyzer should likely be regarded before application. The present study reveals that the reference cutoff values of MCV< 80 fL and MCH < 27 pg could be used for the Coulter DxH500, Coulter LH780, Dirui BF 6800, Pentra ES60, Pentra XL80, Cell-Dyn

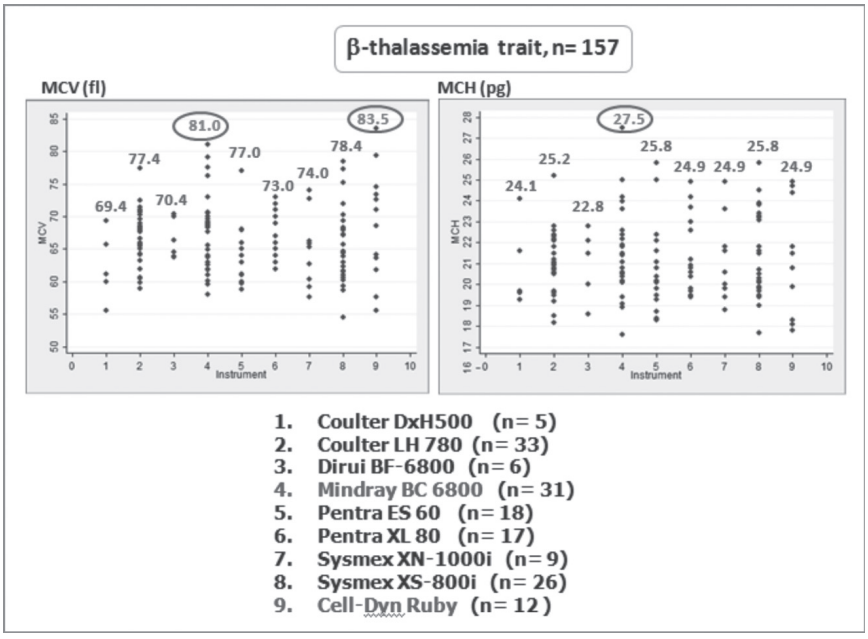

Fig. 1 The MCV and MCH values of β–thalassemia trait (n=157) were analyzed by 9 different hematology analyzers.

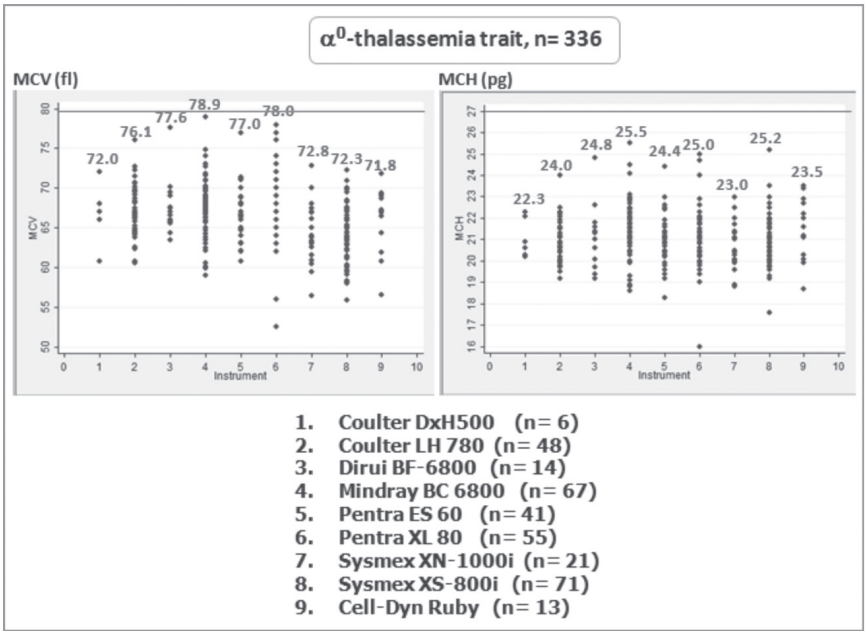

Fig. 2 The MCV and MCH values of α^0–thalassemia trait (n=336) were analyzed by 9 different hematology analyzers.

Ruby, Sysmex XN- 1000i, and Sysmex XS-800i but only one out of 31 samples of β^+-thalassemia trait with MCV > 80 fL and MCH > 27 pg was detected by Mindray BC6800. In order to define these results, retrospective internal quality control of the hematology analyzer was carried out at the community hospital. The data showed that the technician runs the 3 controls of low, normal, and high levels every morning prior to the release of patient results. The control values were shown to be within acceptable limits and the regular maintenance of the equipment was performed. In addition, thalassemia screening protocol in this laboratory does not follow the national guideline. The protocol consisted of the combined one tube osmotic fragility test (OF test), MCV and MCH, and DCIP test. The blood sample in question gave positive result with OF test and was allowed to continue for further investigation. However, this does not indicate that the cutoffs of MCV < 80 fL and MCH < 27 pg are not suitable for Mindray BC6800 machine because a previous study showed that a combined MCV, MCH, and DCIP protocol and a combined OF and DCIP protocol produced the same results of 100% sensitivity and 100% negative predictive value. No false negative results for 3 target forms of α^0-thalassemia, β-thalassemia and Hb E could be detected with those protocols.[9] Moreover, only one sample might not represent significant data. This could be due to machine errors during the day the sample was performed. The limitation has been recognized and our inferences would have been stronger had we determined prospectively RBC indices using the 9 different hematology analyzers with the same specimens and equal sample sizes. In addition, one other case of β-thalassemia trait was found, using Cell-Dyn Ruby, to be 83.5 fL for MCV and 24.7 pg for MCH similar to the results reported by other investigators.[13]

For Hb E screening, many Hb E carriers were found to have MCV above 80 fL. Thus, the national guideline consisting of a combination of MCV and MCH with the DCIP test instead of MCV only was used. It was found that approximately 10.7% of the Hb E cases using KKU-DCIP-Clear test would have been false negative results which were different from previous findings that the KKU-DCIP-Clear test for screening Hb E had 100% negative predictive values.[9] To clarify the findings, we visited the work at the site of the 8 hospitals. One volunteer laboratory staff member from each hospital was tested on their knowledge of Hb E screening and problems related to the screening procedure by questioning and observation during practices. The major factor affecting the misinterpretation was identified to be the laboratory personnel; 4 participants were defined as lacking knowledge of the principle and precautions of the test and 6 participants lacked the skill to read by naked eye. In addition, 6 hospitals were defined as not having positive and negative control samples coexisting with the testing samples.

On the other hand, the 2 hospitals that have internal quality control used homozygous Hb E as a positive control instead of heterozygous Hb E. These findings were similar to the results obtained by Wanthong *et al.* that the false negative results of Hb E screening were due to insufficiency of the quality control system.[14]

Conclusions

Based on these results, using the combined test of blood indices and Hb E screening for pregnant women and their partners following a national screening program for prevention of thalassemia, all clinical laboratories should be concerned over false negative results. Personnel who are responsible for the screening should be retrained and a quality control system should be established, especially, quality control for Hb E screening is needed to standardize and maintain the quality of screening.

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References

1. Fucharoen S, Winichagoon P. Thalassemia in Southeast Asia: problems and strategy for prevention and control. Southeast Asian J Trop Med Public Health 1992; 23: 649-55.
2. Voramongkol N. Prevention and control thalassemia. In: Department of Health, editor. Proceeding of the 7th meeting of National Thalassemia; 2001 June 27-9 Bangkok. Bangkok: Thammasat University; 2001.
3. The Thalassemia Working Party of the BCSH General Haematology Task Force. Guideline for investigation of the α- and β-thalassemia traits. J Clin Pathol 1994; 47: 289-95.
4. A Working Party of the General Haematology Task Force of the British Committee for Standards in Haematology. The laboratory diagnosis of haemoglobinopathies. Br J Haematol 1998; 101: 783-92.
5. Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassemia and haemoglobin E in rural communities in south-east Asia. Bull World Health Organ 2004; 82: 364-72.
6. Sharma P, Das R. Cation-exchange high-performance liquid chromatography for variant hemoglobins and HbF/A2: What must hematopathologists know about methodology? World J Methodol 2016; 6: 20-4.
7. Pornprasert S, Phusua A, Suanta S, Saetung R, Sanguansermsri T. Detection of alpha-thalassemia 1 Southeast Asian type using real-time gap PCR with SYBR Green 1 and high resolution melting analysis. Eur J Haematol 2008; 80: 510-4.

8. Saetung R, Ongchai S, Charoenkwan P, Sanguansermsri T. Genotyping of beta-thalassemia trait by high resolution DNA melting analysis. Southeast Asian J Trop Med Public Health 2013; 44: 1055-64.
9. Sanchaisuriya K, Fucharoen S, Fucharoen G, *et al*. A reliable screening protocol for thalassemia and hemoglobinopathies in pregnancy: an alternative approach to electronic blood cell counting. Am J Clin Pathol 2005; 123: 113-8.
10. Tritipsombat J, Sanchaisuriya K, Phollarp P, *et al*. Micromapping of thalassemia and hemoglobinopathies in different regions of northeast Thailand and Vientiane, Lao PDR. Hemoglobin 2012; 36: 47-56.
11. Jopang Y, Mernkratok S, Puangplruk R. The efficiency of thalassemia and Hb E screening in first-trimester pregnant women at Health Promoting Hospital, Regional Health Promotion Center 5, Nakhon Ratchasima. J Med Tech Assoc Thailand 2004; 32: 585-93. (in Thai)
12. Aslan D. "Silent" β-thalassemia mutation (promoter nt-101 C>T) with increased hemoglobin A_2. The Turkish J of Ped 2016; 58: 305-8.
13. Chaitripop C, Sanchaisuriya K, Inthavong S, *et al*. Thalassemia screening using different automated blood cell counters: consideration of appropriate cutoff values. Clin Lab 2016; 62: 545-52.
14. Wanthong S, Fucharoen G, Sakoopan C, Sanchaisuriya K, Sae-ung N, Fucharoen S. Implementation of quality control system for improvement of thalassemia screening at Maung Saung hospital, Roiet province. J Med Tech Phys Ther 2007; 19: 148-66. (in Thai)