Low Cost Combination of DCIP and MCV Was Better Than That of DCIP and OF in the screening for Hemoglobin E

Kannadit Prayongratana MD*,
Chantana Polprasert MD*, Kasem Raungrongmorakot MD**,
Kanyarat Tatone BSc***, Somchai Santiwatanakul PhD***

** Department of Medicine, Faculty of Medicine, Srinakharinwirot University, Bangkok*
*** Department of Obstetrics and Gynecology, Faculty of Medicine, Srinakharinwirot University, Bangkok*
****Department of Pathology, Faculty of Medicine, Srinakharinwirot University, Bangkok*

Objective: *To evaluate hemoglobin E screening tests in a large scale of cases.*
Material and Method: *A cross-sectional descriptive study was conducted. Whole blood obtained from subjects was evaluated for CBC, OF, DCIP, and hemoglobin typing.*
Results: *Five hundred twenty seven hemoglobin E and 280 reference subjects participated. DCIP's sensitivity, specificity, positive predictive value, and negative predictive value were 97.16%, 98.93%, 99.42%, and 95.19%, respectively. These values of OF were 69.12%, 80.00%, 86.67%, and 57.88%, respectively. In the combination of DCIP and OF gave rise to these values of 99.43%, 79.29%, 90.03%, and 96.67%, respectively. Finally the combination of DCIP and MCV < 80 fL resulted in these values to be 99.43%, 98.93%, 99.43%, and 98.93%, respectively. False positive and false negative rate were 1.07% and 0.57%, respectively.*
Conclusion: *Combination of DCIP and MCV was better than that of DCIP and OF in hemoglobin E screening.*

Keywords: *Thalassemia, Heterozygous hemoglobin E, Homozygous hemoglobin E, Dichlorophenolindolphenol test (DCIP), Osmotic fragility test (OF), Mean corpuscular volume (MCV)*

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Thalassemia is the most important genetic abnormality in Thailand, which has the highest gene frequency of thalassemia in the world. Thirty-seven percent of the Thai population has the thalassemia and hemoglobinopathy gene, and a thalassemia patient is approximately 1% of its population[1,2]. Many types of gene mutation were found.

Hemoglobin E (Hb E) is the most frequent abnormal hemoglobin among the Thai population. It is not only a hemoglobinopathy but also a thalassemia. Hemoglobin E results from mutation of the twenty-sixth codon of β-globin gene and results in substitution of glutamic acid with lysine. This mutation activates cryptic splice site at the 25th codon and results in shortage of the β-globin chain, which is the cause of non-functioning β-globin chain. This chain cannot couple with the α-globin chain and thalassemia or quantitative globin defect also occur[3]. The gene frequency of Hemoglobin E in Thailand varies depending on study location from 12.6 to 50%[1,2,4-11]. This high frequency was found along the Thailand-Cambodia border in Northeastern and eastern regions of Thailand[1,2,4,5].

The impact of problem is that Hemoglobin E carriers and homozygous Hb E people can produce diseased off spring if they get married with β-thalassemia carriers. The Hb E/β-thalassemia compound heterozygotes are the most abundant form of Thalassemia disease in Thailand[1,2,4,5]. The population growth data from the Department of Provincial Administration, Ministry of Interior of Thailand[12] and Wong et al[11] estimate that 1,600 cases per year of Hb E/β-thalassemia patients will be born. To limit this problem,

Correspondence to: Prayongratana K, Division of Hematology, Department of Medicine, Faculty of Medicine, Srinakharinwirot University, 62 Moo 7, Rangsit-Nakhonnayok Rd, Ongkharak, Nakhonnayok 26120, Thailand. Phone: 037-395-085-6 ext 11001, Fax: ext 11003. E-mail: kannadit_swu@yahoo.com

preconception carrier detection and selected prenatal diagnosis are both important.

Two tests that consist of one tube osmotic fragility (OF) test and dichlorophenolindolphenol (DCIP) test are routinely applied to screen the Hb E cases. The former test is assigned to detect the hypochromic microcytic red cells, which decrease the fragility of erythrocytes because they have more space for the hypotonic solution than that of the normal erythrocytes have. The latter test is used to detect the presence of HbE which is easily and quickly oxidized by DCIP reagent and is then precipitated[13]. The present study was performed to evaluate both of these tests with a large scale of samples.

Material and Method

The present study was approved by the ethic committee for the studies in humans in accordance with the Helsinki declaration. These participants attended Her Royal Highness Princess Mahachakri Sirindorn Medical center (MSMC) hospital, Faculty of Medicine, Srinakharinwirot University.

Between September 2004 and August 2007, 853 cases of Hemoglobin E, which included Hemoglobin E heterozygote and homozygote were diagnosed by using hemoglobin typing. Among this population, only 527 cases were completely evaluated for complete blood count (CBC), one tube OF test, DCIP test, and hemoglobin analysis and were recruited. The 280 reference population that had hemoglobin concentration not less than 10 g/dL, normal mean corpuscular volume; MCV (range 80-100 fL[14]), and normal hemoglobin typing were recruited as control or reference population group. CBC was done by using Sysmex XT-2000*i* automated hematology analyzer[15]. CBC data including hemoglobin concentration, hematocrit, red blood cell indices, and red blood cell distribution width (RDW) were collected. OF and DCIP were easily performed by using KKU-OF™ and KKU-DCIP™ reagent kits that were developed by Fucharoen S, Faculty of Medical Technology, Khon Kaen University, Thailand[10]. Hemoglobin analysis was performed by using high performance liquid chromatography (HPLC) with the VARIANT-II™, Bio-Rad Laboratory.

OF was performed by adding 20 μL of the patient's whole blood to 2 mL of 0.36% NaCl solution, then left at room temperature for at least 15 minutes and interpreted. Positive OF test or decreased fragility of erythrocytes was shown as the turbid solution which contained non-hemolysis of hypochromic microcytic erythrocytes; whereas, negative OF test was shown as a clear red solution that contained complete hemolysis of normochromic normocytic erythrocytes.

DCIP was performed by adding 20 μL of the patient's whole blood to the DCIP solution and warmed at 37°C for 15 minutes, then added the clearing reagents and left at room temperature for at least 5 minutes and interpreted. Positive DCIP test was shown as a turbid brown solution that contained oxidized hemoglobin; whereas, negative DCIP test remained as clear blue.

Statistical analysis

The present study design was a descriptive cross-sectional study. The baseline characteristics between hemoglobin E group and reference population group were compared by using SPSS for Windows® version 11.0. Continuous data, which consisted of age, hemoglobin, hematocrit, MCV, MCH, MCHC and RDW, summarized with mean $\pm$ standard deviation (mean $\pm$ SD), were compared by using the independent t-test. Sex, which was a discrete data, was compared by using Chi-square test. The OF and DCIP were tested for sensitivity, specificity, positive predictive value, negative predictive value, false positive rate, and false negative rate. A p-value of less than 0.05 was considered statistically significant.

Results

Between September 2004 and August 2007, 853 cases of Hemoglobin E heterozygote and homozygote were diagnosed. Among this population 539 cases were completely evaluated but 12 of them were excluded from the study because their hemoglobin was too low to be explained by carrier state alone. Therefore, only 527 cases were tested for complete blood count, one tube OF test, DCIP test, and hemoglobin analysis. The 280 reference population that had hemoglobin concentration at least 10 g/dL, normal MCV, and normal hemoglobin typing were assigned as control or reference population group. These populations were also tested the same as the patients. The baseline characteristic of hemoglobin E group and reference group are summarized in Table 1.

The DCIP test was performed in the hemoglobin E heterozygote and homozygote patients (HbE group) and reference group. Five hundred and thirteen of 527 patients (97.34%) were positive and 14 patients (2.66%) were falsely negative. In the reference population, three of 280 cases (1.07%) were falsely positive and 277 cases (98.93%) were negative. The sensitivity and specificity were 97.16% and 98.93%,

Table 1. Patient's and reference population characteristics

Variable	Hemoglobin E group (n = 527)	Reference group (n = 280)	p-value
Age (year), mean ± SD	28.46 ± 7.80[a]	28.31 ± 7.13	0.796 (-0.93 to 1.24)[c]
Sex			0.002
Male (%), n	197[b] (37.38)	137 (48.93)	
Female (%), n	330 (62.62)	143 (51.07)	
Hemoglobin (g/dL), mean ± SD	12.59 ± 1.59	13.36 ± 1.72	<0.001(-1.01 to -0.52)
Hematocrit (%), mean ± SD	37.09 ± 4.74	39.52 ± 4.94	<0.001(-3.14 to -1.72)
MCV (fL), mean ± SD	74.56 ± 6.81	84.87 ± 4.31	<0.001(-11.08 to -9.54)
MCH (pg), mean ± SD	25.34 ± 2.29	28.70 ± 1.69	<0.001(-3.64 to -3.08)
MCHC (g/dL), mean ± SD	33.97 ± 0.99	33.69 ± 2.07	0.031(0.03 to 0.54)
RDW (%), mean ± SD	14.77 ± 1.70	13.63 ± 0.84	<0.001(0.97 to 1.32)

a, mean ± SD
b, number
c, 95% confidential interval of mean difference

Table 2. Results of DCIP and OF tests

	DCIP	OF	DCIP + OF	DCIP + MCV
Sensitivity	97.16%	69.12%	99.43%	99.43%
Specificity	98.93%	80.00%	79.29%	98.93%
Positive predictive value	99.42%	86.67%	90.03%	99.43%
Negative predictive value	95.19%	57.88%	96.67%	98.93%
False positive rate	1.07%	20.00%	20.71%	1.07%
False negative rate	2.66%	-*	0.57%	0.57%

* Heterozygous hemoglobin E's erythrocytes could be normocytic or microcytic, so OF could be both positive and negative

respectively. Positive predictive value, and negative predictive value were 99.42%, and 95.19%, respectively (Table 2).

The OF test was performed in the hemoglobin E heterozygote and homozygote and reference group. Three hundred and sixty four of 527 patients (69.07%) were positive and the 163 cases (30.93%) were negative. In the reference population, 56 cases (20%) were falsely positive and 224 cases (80%) were negative. The sensitivity and specificity were 69.12% and 80.00%, respectively. Positive predictive value and negative predictive value were 86.67% and 57.88%, respectively (Table 2).

Combined DCIP and OF also evaluated which revealed that sensitivity, specificity were 99.43% and 79.29%, respectively. Positive predictive value and negative predictive value were 90.03% and 96.67%, respectively. False positive and false negative rate were 20.71 and 0.57, respectively (Table 2).

Finally, the combination of DCIP, and MCV showed that sensitivity, specificity were 99.43% and 98.93%, respectively. Positive predictive value and negative predictive value were 99.43% and 98.93%, respectively. False positive and false negative rate were 1.07% and 0.57%, respectively (Table 2).

Discussion

Hemoglobin E is the most common hemoglobinopathy in Southeast Asia. Most of the data of these genetic abnormalities came from Thailand because the peak of gene frequency of this hemoglobinopathy in the world is in Southeast Asia[1,2]. Due to the worldwide migration of the population including the Hb E heterozygote and homozygote from this region of the world causes the problem. The impact of the problem is that, if the Hb E containing population get married with b-thalassemia carrier or sickle cell trait or patients that are more prevalent in the West, the compound

heterozygote patient will occur. Data from western countries were very few and population included in the present study was small[16]. This may result from the limitation of rarity of the cases. Therefore, the present paper aimed to support the confidence of using DICP and OF tests.

From the large impact of this problem in Thailand, test kits were developed to screen for presence of Hb E. Many papers have reported the effectiveness of using DCIP and found that this test has a sensitivity of 94.4-100%, specificity of 69.8-98.2%, positive predictive value of 75.0-86.9%, and negative predictive value of 98.1-100%[13,16,17]. The present study also found that DCIP was a good screening test with sensitivity and specificity of 97.16% and 98.93%, respectively. Positive predictive value and negative value were 99.42% and 95.19%, respectively. The false positive and the false negative rates were low which were 1.07% and 2.66%, respectively.

The sensitivity and the negative predictive value of OF was quite low (69.12% and 57.88%, respectively). The specificity and positive predictive value were acceptable (80% and 86.67%, respectively). The false positive rate was as high as 20%. Presently, no study has reported the use of OF test alone to screen the HbE group but usually combined with DCIP[17-19]. This may result from the poor prediction of OF in the screening of HbE group as reported in the present study. To confirm the previous studies, the authors also analyzed the combination of OF and DCIP for screening these cases.

The combination of OF with DCIP increased the sensitivity and negative predictive value when compared with DCIP and OF alone (99.43% and 96.97%, respectively). This combination had lower specificity and positive predictive value (79.29% and 90.03%, respectively) when compared to DCIP alone. These results of both tests combined were comparable to previous studies which reported the sensitivity, specificity, positive predictive value and negative predictive value of 100%, 79.7-97.1%, 84.5-94.9%, and 100%, respectively[17-19].

Finally, at present there is widespread use of mean corpuscular volume (MCV) in modern automated CBC analyzers to screen HbE group. Most of the MCV of HbE group, carrier and patients, were usually low but some cases of Hb E heterozygote might have normal MCV. The present study found that 431 cases (81.82%) of hemoglobin E cases had MCV less than 80 fL. The combination of DCIP and MCV further increased the sensitivity, positive predictive value, and negative predictive value while the specificity and false positive rate were equally when compared to DCIP alone. Furthermore, this combination increased all of these values when compared to the combination of DCIP and OF. Although there was no previous data of this combination, the present study discovered that this combination is the most appropriate screening test for the hemoglobin E cases.

Conclusion

DCIP is useful in the screening of Hemoglobin E heterozygote and homozygote with high sensitivity, specificity, positive predictive value, and negative predictive value. Moreover, it had low false negative and false positive rate. OF had low sensitivity and negative predictive value, with acceptable specificity and positive predictive value. MCV<80 fL was better than OF in this situation. Combination of DCIP and MCV showed that it is better than that of DCIP and OF in hemoglobin E screening because it increased the sensitivity, positive predictive value, and negative predictive value. Furthermore, this combination also decreased the false negative rate when compared with DCIP alone and DCIP-OF combination.

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การคัดกรองพาหะธาลัสซีเมียชนิดฮีโมโกลบิน อี โดยใช้ DCIP ร่วมกับ MCV ดีกว่าการใช้ DCIP ร่วมกับ OF

กานดิษฎ์ ประยงค์รัตน์, จันทนา ผลประเสริฐ, เกษม เรืองรองมรกต, กันยารัตน์ ทาโทน, สมชาย สันติวัฒนกุล

วัตถุประสงค์: *เพื่อเปรียบเทียบการตรวจคัดกรองสำหรับพาหะธาลัสซีเมียชนิดฮีโมโกลบิน อี ว่าชุดการตรวจใดมีความแม่นยำมากกว่า*

วัสดุและวิธีการ: *การศึกษาเชิงพรรณนาภาคตัดขวางทำการศึกษาในผู้ป่วยอายุรศาสตร์ และคู่สมรสที่มาตรวจที่คลินิกวางแผนครอบครัวที่โรงพยาบาลศูนย์การแพทย์สมเด็จพระเทพรัตนราชสุดาฯ ระหว่าง กันยายน พ.ศ. 2547 ถึง สิงหาคม พ.ศ. 2550 โดยเก็บข้อมูลจากการตรวจ CBC, OF, DCIP และ hemoglobin typing*

ผลการศึกษา: *มีพาหะธาลัสซีเมียชนิดฮีโมโกลบิน อี 527 ราย และคนปกติ 280 รายเข้าร่วมการศึกษา พบว่าค่าความไว, ความจำเพาะ, positive predictive value และ negative predictive value ของ DCIP เป็นร้อยละ 97.16, 98.93, 99.42 และ 95.19 ตามลำดับ ค่าดังกล่าวสำหรับ OF เป็นร้อยละ 69.12, 80.00, 86.67 และ 57.88 ตามลำดับ หากใช้ DCIP ร่วมกับ OF พบว่าค่าดังกล่าวเป็นร้อยละ 99.43, 79.29, 90.03 และ 96.67 ตามลำดับ และถ้าใช้ DCIP ร่วมกับ MCV ที่น้อยกว่า 80 fL พบว่าค่าดังกล่าวเป็นร้อยละ 99.43, 98.93, 99.43, และ 98.93 ตามลำดับผลบวกลวงเป็นร้อยละ 1.07 และผลลบลวงเป็นร้อยละ 0.57*

สรุป: *DCIP ร่วมกับ MCV เป็นการตรวจที่ดีกว่า DCIP ร่วมกับ OF สำหรับการคัดกรองพาหะของฮีโมโกลบิน อี*