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ORIGINAL ARTICLE

Potential use of cord blood for Hb E hemoglobinopathy screening programme using capillary electrophoresis

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Abstract

Background: Thalassemia and hemoglobinopathies are inherited red blood cell disorders found worldwide. Hemoglobin (Hb) E disorder is one of the hemoglobinopathies known to have the high prevalence in South East Asia. Most of transfusion-dependent thalassemias were genotypically compound heterozygous Hb E/ β-thalassemia. In Malaysia, the national screening program for thalassemia was implemented for early pregnancy or secondary school girls; however many participants do not turn-up and missed the screening test. Screening for thalassemia using samples from cord blood is an alternative choice as it is a readily available source of blood and hence early detection of the disease. The purpose of this study was to determine the potential use of cord blood for the screening of HbE hemoglobinopathy by using capillary electrophoresis (CE). *Methods:* Cord blood samples were collected from 300 newborns of healthy mothers. Hematological parameters were determined and hemoglobin quantitation for all cord blood samples were performed using capillary electrophoresis system (CES) and high performance liquid chromatography (HPLC). *Results:* Majority of cord blood samples (63%) revealed Hb AF followed by Hb AFA_2 (20%). Hb AFE was detected in 10.7% with the mean value of Hb E ranging from 2.3%-11.1%. *Conclusion:* Hemoglobin E was detected in cord blood using capillary electrophoresis system. It can be recommended in areas where Hb E/β is prevalent. Implementation of a screening strategy using CE on cord blood sampling will identify the disease early. With regular follow-up on these patients, the status of their disease can be determined earlier and appropriate management implemented.

Keywords: cord blood screening, capillary electrophoresis, thalassemia

INTRODUCTION

Thalassemia is an inherited autosomal recessive disorder characterized by complete absence or decrease in the production of either alpha (α) or beta (β) globin gene. Hemoglobinopathy refers to an inherited structural abnormality of globin genes that will lead to abnormal hemoglobin synthesis. Hb E is a variant hemoglobin with a mutation in the β globin gene causing substitution of glutamic acid for lysine at position 26 of the β globin chain. Hb E syndromes or Hb E disorders are heterogeneous groups of hemoglobinopathies whose phenotype range from asymptomatic to severe. Hb E trait and Hb EE are mild disorders whereas Hb E/ β-thalassemia results in severe transfusion dependent thalassemia.

In Southeast Asian countries, β-thalassemia, hemoglobin E and β-thalassemia are prevalent[1] with the frequency of α-thalassemia ranging 4-40% and β-thalassemia 1-9%. Hemoglobin E is the disease hallmark of Southeast Asia with a frequency of 50-60% in areas between Thailand, Laos and Cambodia.[2] Malaysia, which is located south of Thailand, has a heterogeneous population comprising 65% Malays, 26% Chinese and 8% Indians. About 4.5% of Malaysians are heterozygous carriers for β-thalassemia.[3,4] Other types of Thalassemias carrier among blood donors were Hb E/ α-thalassemia (38.5%), Hb E / β-thalassemia (23.1%), Hb E trait (7.6%) and β-thalassemia (30.8%).[5] The risk of producing a child with β-thalassemia major annually

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was estimated to be 2.1/1,000. Based on the Malaysian Thalassemia Registry (2010), there were 4768 transfusion dependent thalassemia.[6] Screening for thalassemia in Malaysia include voluntary screening for carriers among school children followed by genetic counseling.[6] There are also prenatal diagnosis facilities with an option for termination of pregnancy, if the fetus is found to have Thalassemia major. With due respect of Islamic religious practices by 60% of Malaysian population, pertaining to the termination of pregnancy, the Ministry of Health with the National Fatwa Council Malaysia have ruled that a pregnancy can be terminated before 120 days of gestation if a severe disorder is confirmed in the fetus.[7]

In Malaysia, cord blood screening for congenital hypothyroidism and Glucose 6-phophate dehydrogenase (G6PD) deficiency are routinely carried out as part of newborn national screening programme. In addition to the existing cord blood investigations, the implementation of cord blood screening for thalassemia would be feasible and may overcome one of the common health burdens in Malaysia, that of late detection of Hb E disorders.

The choice of screening method depends on the availability of resources. Red blood cell indices remain the first line test for thalassemia work-up. In some countries, osmotic fragility testing is implemented as a population screening tool.[8] Many laboratories use high performance liquid chromatography (HPLC) for the β-thalassemia screening program. The limitation of this system is Hb E co-eluates with Hb A_2 thus samples from cord blood or newborn requires further testing.

Capillary electrophoresis (CE) has been introduced as an alternative tool that is capable of separating the hemoglobins accurately. In many western countries, newborns screening for hemoglobinopathies has been recommended as a tool for morbidity prevention. Cord blood sampling is less invasive and more readily accepted by parents for screening purposes. Thus, the aim of this study was to determine the potential use of cord blood for the screening of HbE hemoglobinopathy using capillary electrophoresis (CE).

MATERIALS AND METHODS

Subjects and hematological analyses

This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (USMKK/PPP/JEPeM [246.3.(5)]). This cross-sectional study was done by collecting 300 cord blood samples from newborns delivered at Hospital Universiti Sains Malaysia (HUSM). Informed consent was obtained from the mothers before delivery. Preterm newborns and newborns whose mothers were having medical or hematological problems were excluded from the study. Five ml of liquid cord blood samples were collected in ethylenediaminetetraacetate (EDTA). Care was taken to avoid contamination with maternal blood. Hematological parameters of hemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were measured using the Sysmex XE 5000 (Sysmex, Kobe, Japan) analyzer. The liquid cord blood was readily analyzed by HPLC.

Hemoglobin quantitation of all cord blood samples for Hb A, Hb F, and Hb A_2, Hb Bart's and Hb E were done using capillary electrophoresis (CE) system (CAPILLARYS 2 Sebia, Lisses, France). For hemoglobin quantitation using CE, the anticoagulated cord blood was centrifuged at 5000 rpm for 5 minutes, the plasma was discarded and the packed red blood cells were washed for two times with ten volume of saline before being analyzed by CE.

Hemoglobin analysis was also performed on high performance liquid chromatography (HPLC) (Variant II Beta Thalassemia Short Program, Bio-Rad Laboratories). The anticoagulated liquid cord blood was readily analyzed by HPLC.

DNA analyses

DNA extraction was prepared from liquid cord blood leukocytes using the NucleoSpin® method. The β^E mutation were identified using GenoFlow Beta Thalassemia Array Test Kit (Southeast Asia, Diagcor Bioscience Incorporation Limited).

Statistical analyses

Descriptive statistics including mean and standard deviation were used to describe haematological parameters and haemoglobin profiles of all subjects. Correlation study was performed for the quantitation of Hb F, Hb A_2 and Hb E using CE and HPLC.

RESULTS

The characteristic of samples were shown in Table 1. The mean age of newborns was 38.8±1.5 weeks. The majority (96.7%) of the samples were from Malays. All had normal serum ferritin.

Table 2 shows the hemoglobin profiles of cord blood using both CE and HPLC. Hb A and Hb F were the main types of hemoglobin in cord blood.

TABLE 1: Demographic and laboratory features of the newborns screened

Characteristic	Frequency n (%)	Mean ± SD (Range)
Gestational age in weeks		38.8 ± 1.5 (38.70 – 39.03)
Ethnicity		
Malays	290 (96.7%)	
Thai	6 (2.0%)	
Chinese	3 (1.0%)	
Others	1 (0.3%)	
Gender		
Male	160 (53.3%)	
Female	140 (46.7%)	
Birthweight in kg		3.06 ± 0.45 (3.01 – 3.11)
Hemoglobin in g/dL		15.35 ±1.65 (10.50 – 20.00)
MCV in fL		102.2 ± 5.4
MCH in pg		23.0 ± 2.1

TABLE 2: Hemoglobin profiles of cord blood assessed by capillary electrophoresis (CE) and HPLC

Hb Type	n	Capillary Electrophoresis				HPLC		
		Hb A (%) Mean (SD)	Hb F (%) Mean (SD)	Hb A2 (%) Mean (SD)	Hb E (%) Mean (SD)	Hb A (%) Mean (SD)	Hb F (%) Mean (SD)	Hb A_2 (%) Mean (SD)
A/F	189	15.3 (4.4)	84.6 (4.4)	–	–	16.8	83.1+6.7	–
A/F/A^2	60	25.5 (6.9)	73.0 (6.3)	0.3 (0.2)	–	26.4	72.2 (8.5)	0.46 (0.7)
A/F/E	32	9.9 (4.9)	85.2 (6.9)	–	4.7 (1.9)	11.7 (5.1)	83.4 (6.6)	4.8 (2.7)
*A/F/Bart's	16	16.2 (7.2)	82.5 (7.5)	–	–	18.8 (7.3)	81.1 (7.3)	–
*A/F/A2/Bart's	1	29.1	69.9	0.3	–	33.1	66.9	–
*A/F/E/Bart's	1	9.2	86.6	–	3.7	11.4	84.4	4.3

* Data Hb Bart's was not presented

**One sample was identified as group AFS by both CE and HPLC but data not presented

The majority (63%; 189 cases) were grouped as AF, followed by AFA_2 (20%; 60 cases). By the CE method, some cord blood samples showed a trace amount of Hb A_2. Those with Hb E peak did not exhibit Hb A_2. Thirty-two cases (10.67%) with Hb AFE type showed a significant amount of Hb E with mean of 4.7 ± 1.9% by CE. Samples that demonstrated Hb E by CE had higher Hb A2 mean levels measured by HPLC (Hb A_2 4.8 ± 2.7%). There were two samples having a unique combination of hemoglobins which were Hb AFA_2Bart's and Hb AFEBart's. Similar hemoglobin groups were detected by HPLC with no statistically difference in the quantitative level of hemoglobin types (Table 2). All samples with Hb E peak were also positive for codon 26 (GAG - > AAG).

A correlational study was done for quantitation of Hb F and Hb E between these two methods. There was good correlation of Hb F level with r value of 0.821 (Fig. 1a). Both methods showed almost similar levels of Hb F: 82.13 ± 7.19% for CE and 80.77 ± 7.18% by HPLC ($p > 0.05$).

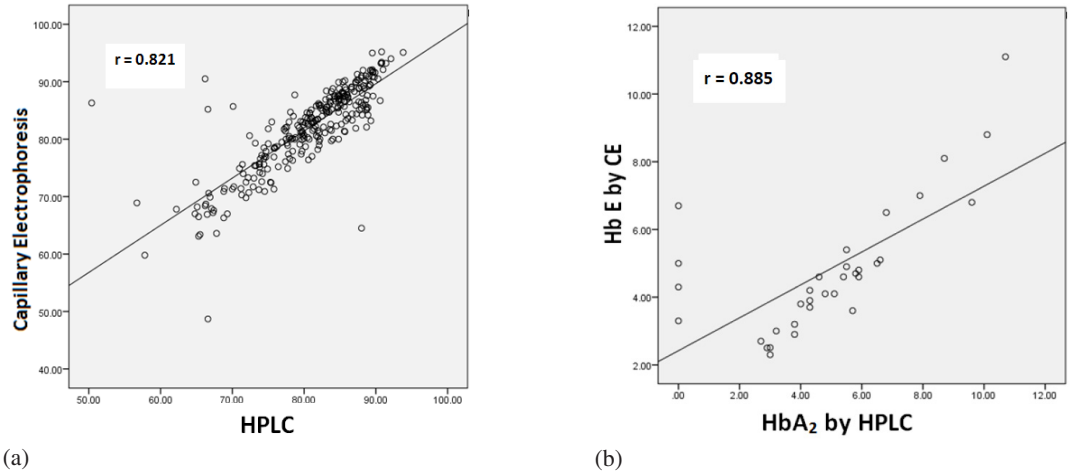
Hb E peak was identified by CE and quantitatively measured. There was good correlation for measurement of Hb E by CE and measurement of Hb A2 by HPLC (r = 0.885) (Fig. 1b). The mean value of Hb E was 4.78 ± 1.95% and Hb A_2 was 4.86 ± 2.73% for CE and HPLC respectively.

DISCUSSION

The hemoglobin group AFE was the third highest hemoglobin groups in this study, accounting for 32 cases (10.7%). One sample with Hb AFEBart's with a mean value of Hb E of 4.7 ± 1.9 % (range: 2.3 - 11.1%) was encountered. All of these samples were positive for codon 26 (GAG - > AAG) by molecular testing using GenoFlow Beta Thalassemia Array Test Kit (Southeast Asia). Hb A was detected in all of these newborns ranging from 3.5 - 24.7%. The presence of Hb A in these groups indicated that none of them will be Hb E/β^0 or Hb E disease later in life. The prevalence of Hb E in our study corresponds with the prevalence of Hb E in Malaysia (5 - 46%).[6]

A study among newborns using CE in Thailand reported that the highest level of Hb E was observed in newborns with pure homozygous Hb E ($\alpha\alpha/\alpha\alpha$, $\beta^E\beta^E$); 8.3% to 13.2% in newborns with FE Hb type and 13.2% with FA_2E Hb type. A lower Hb E level was observed in newborns with heterozygous Hb E ($\alpha\alpha/\alpha\alpha$, $\beta^E\beta^A$) with a mean value of 5.1 ± 2.3% in newborns with AFA_2E Hb type and 3.0 ± 1.1% with AFE Hb type.[9]

There are few initial methods to screen for thalassemia or hemoglobinopathy among newborn such as HPLC, isoelectric focusing (IEF), and capillary electrophoresis. These methods are preferred over cellulose acetate agar electrophoresis because they are less labour intensive and have high throughput. In this study both CE and HPLC were used as the screening tools. They provide quantitatively similar levels for all hemoglobin subtypes ($p > 0.05$). The correlation study by CE and HPLC for Hb F and Hb A_2/E were r = 0.821 and 0.885 respectively. By using CE, Hb E peak was eluted at a different retention time to Hb A_2 making the presumptive diagnosis of Hb E syndrome straight forward.



(a) (b)

FIG. 1: Correlation of (a) Hb F and (b) Hb E by CE and HPLC

However, using HPLC, Hb A_2 and Hb E was non-separable and a cutoff point was required to differentiate the significant level of Hb A_2E for the diagnosis of Hb E syndrome. Analysis of Hb E for Hb E related disorders using CE could make the diagnosis with more certainty as the CE was able to separate Hb E from Hb A_2. In addition, CE gave excellent accuracy for identification of Hb E in newborns as all samples were positive for codon 26 (GAG - > AAG).

The cost for hemoglobin analysis by CE was RM24.50 per test, which was slightly more expensive than the cost for hemoglobin analysis by HPLC (RM22.50). The duration of sample preparation, performing analysis for control, and the duration from loading the sample into instrument until production of the results for both instruments were almost the same, which was about 40 minutes.

Based on our findings, thalassemia screening using cord blood is implementable. Hb E related disorders were common types of hemoglobinopathy in South East Asia and this hemoglobin can be easily detected and discriminated by CE unlike the existing HPLC method which requires additional testing to confirm Hb E. CE is one of the most powerful first-line solutions for thalassemia screening in newborns. Our results demonstrated that the capillary electrophoresis system which was developed for hemoglobin fraction separation and quantification can give good performance comparable to conventional HPLC for quantification of Hb A and Hb F when used in newborn screening for thalassemia.

In conclusion, the implementation of a screening strategy using CE on cord blood samples in areas where Hb E hemoglobinopathy is prevalent, is highly recommended as it is feasible and the disease is detected early. Regular follow-up of these patients will identify those that may become transfusion-dependent and appropriate management will improve the disease outcome and social burden to their families and the country.

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