Malaysian J Pathol 2017; 39(1) : 17 – 23

ORIGINAL ARTICLE

Detection of α-thalassaemia in neonates on cord blood and dried blood spot samples by capillary electrophoresis

Hafiza ALAUDDIN *MBBS, MPath*, Mustafa LANGA *MBBS, MPath*, Malisa MOHD YUSOFF *Dip MLT*, Raja Zahratul Azma RAJA SABUDIN *MBBS, MPath*, Azlin ITHNIN *MBBchBAO, MPath*, Noor Farisah ABDUL RAZAK* *BSc (HONS)*, Nor Hidayati SARDI* *Dip MLT* and Noor Hamidah HUSSIN *MBBCh, DrMed Sc*

*Haematology Unit, Pathology Department and *Diagnostic Laboratory Services, UKM Medical Centre (UKMMC), Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia*

Abstract

Introduction: Haemoglobin Bart's (Hb Bart's) level is associated with α-thalassaemia traits in neonates, enabling early diagnosis of α-thalassaemia. The study aimed to detect and quantify the Hb Bart's using Cord Blood (CB) and CE Neonat Fast Hb (NF) progammes on fresh and dried blood spot (DBS) specimen respectively by capillary electrophoresis (CE). *Methods:* Capillarys Hemoglobin (E) Kit (for CB) and Capillarys Neonat Hb Kit (for NF) were used to detect and quantify Hb Bart's by CE in fresh cord blood and dried blood spot (DBS) specimens respectively. High performance liquid chromatography (HPLC) using the β-Thal Short Programme was also performed concurrently with CE analysis. Confirmation was obtained by multiplex ARMS Gap PCR. *Results*: This study was performed on 600 neonates. 32/600 (5.3%) samples showed presence of Hb Bart's peak using the NF programme while 33/600 (5.5%) were positive with CB programme and HPLC methods. The range of Hb Bart's using NF programme and CB programme were (0.5–4.1%) and (0.5-7.1%), respectively. Molecular analysis confirmed all positive samples possessed α-thalassaemia genetic mutations, with 23/33 cases being αα/--^{SEA}, four -α$^{3.7}$/-α$^{3.7}$, two αα/-α$^{3.7}$ and three αα/ααα^{CS}. Fifty Hb Bart's negative samples were randomly tested for α-genotypes, three were also found to be positive for α-globin gene mutations. Thus, resulting in sensitivity of 91.7% and 88.9% and specificity of 100% for the Capillarys Cord Blood programme and Capillarys Neonat Fast programme respectively. *Conclusion*: Both CE programmes using fresh or dried cord blood were useful as a screening tool for α-thalassaemia in newborns. All methods show the same specificity (100%) with variable, but acceptable sensitivities in the detection of Hb Bart.

Keywords: neonatal α-thalassaemia screening, α-thalassaemia, capillary electrophoresis, cord blood

INTRODUCTION

α-thalassaemia carriers can be identified with more certainty in the neonatal period, when they have 1-2% Haemoglobin (Hb) Bart's.[1] It disappears over the first few months of life and is not replaced by Hb H. However, its absence does not exclude the diagnosis and confirmatory diagnosis can only be confirmed by genetic mapping.[2]

α-thalassaemia trait is due to the loss of two of the four α-globin genes. It is characterized by a moderate increase of Hb Bart's (5–6%) in the newborn and by α-thalassaemia-like red blood cell indices, which exhibits mild microcytic, hypochromic anaemia with mean cell volume (MCV) between 70-75 fL.[3] In adults, α-thalassaemia trait may be confused with iron deficiency anaemia because of normal HbA_2 and HbF levels as well as absence of Hb Bart's. Detection of α-thalassaemia in newborns is even less straight forward due to the physiologically high Hb and RBC parameters.[4] The condition will usually remain undiagnosed with until much later and it is therefore a great advantage to detect these carriers at birth for better population screening and public health planning.

The measurement of the Hb Bart's in α-thalassaemia newborn screening was to distinguish between Hb H disease and α-thalassaemia minor. British National Screening Committee suggested that all newborns with Hb

Address for correspondence: Noor Hamidah Hussin, Haematology Unit, Pathology Department, UKM Medical Centre (UKMMC), Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Email: hamidah@ppukm.ukm.edu.my

Bart's concentrations of 15% or more required further evaluation, as they might develop Hb H disease. However, at low concentrations (Hb Bart's 1%-8%) were indicative of the carrier states and usually did not need further evaluation. However, these reference ranges vary among practising laboratories.[5]

The analysis of newborn blood adopted the same Hb separation technologies used for adults. The only difference is the main Hb fraction is Hb A ($\alpha_2\beta_2$) in adults, while it is HbF ($\alpha_2\gamma_2$) in newborns.

Qualitative and quantitative haemoglobin analysis by capillary electrophoresis (CE), high-performance liquid chromatography (HPLC) and additional techniques such as isoelectric focusing and citrate agar electrophoresis have been used to identify the quantity and types of Hb relevant to α-thalassaemia (Hb Bart's and Hb H). Identification of abnormal fractions is possible with all these methods at comparable degrees of performance. However, confirmation must be made at the protein or molecular level.[4] Provisional detection of patients and carriers are made possible by the above methods therefore management plan and genetic counselling is made possible not only for those couples at risk who has a child affected with thalassaemia (retrospective primary prevention), but also for those at risk who has a healthy child carrier (prospective primary prevention). In this way 75% of all couples at risk could be possibly be identified.[6]

The preliminary screening of thalassaemia using dried blood spot (DBS) carries less of a biohazard risk as compared to liquid samples, requires minimal storage facilities since the samples are stable at 4°C or room temperature for extended periods (7-10 days), and may easily be sent out to the reference laboratories. The DBS may also provide a cheaper alternative for identifying thalassaemia in regions with increased frequency of carriers. It is also useful in over-populated countries with limited resources.[7]

In this study, we evaluated the use of capillary electrophoresis system (CE) for the diagnosis α-thalassaemia in neonates using their cord whole blood and dried blood forms using Cord Blood (CB) and Neonat Fast Hb (NF) Programmes respectively. We also compared these detection methods with High Performance Liquid Chromatography (HPLC) method.

MATERIALS AND METHODS

The study was approved by the Institution (UKMMC) Ethics Committee. Blood samples were collected in EDTA tubes. Six hundred full term newborn cord blood samples sent to Hematology Laboratory, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) for G6PD screening between July 2013 and March 2014 were used in this study with informed consents from their parents. Samples after 24 hours of collection, clotted and insufficient samples were excluded. Each sample was separated into three portions and prepared accordingly into haemolysate, packed red cell and dried blood forms.

High performance liquid chromatography (HPLC) system

Haemolysates of the samples were analyzed with high performance liquid chromatography (HPLC) using VARIANT™ Hemoglobin Testing system with β-Thalassemia Short Programme (Bio-Rad Laboratories, Hercules, CA, USA) to determine the Hb subtypes as described in the instruction manual. Hb Bart's is eluted at the beginning of chromatogram, at the retention time 0.11 minutes (Fig. 1a).

Capillary zone electrophoresis (CE) system

(a) Cord blood programme

Haemoglobin analysis by cord blood programme was performed on the haemolysate of the packed red cells in alkaline buffer (pH 9.4) using the Capillarys™ (Sebia, Paris, France). The Capillarys Hemoglobin (E) kit (Sebia, Paris, France) was used in the sample preparation and the sample was run according to the manufacturer's instructions. The potential position of the different Hb Bart's is in zone 12 (Fig. 1b).

(b) Neonat Fast Hb programme

Haemoglobin analysis by NF programme, by Capillarys™ (Sebia, Paris, France) using Neonat Hb kit (Sebia, Paris, France) was performed on the haemolysate of dried whole blood samples previously spotted on Guthrie filter paper. Demineralized water was added into the wells of the Capillarys segment for pre-elution of haemoglobin from a 3.8 mmØ dried blood spots on the Guthrie card. Extraction was done directly into the wells of the elution segments, containing 50 µl of distilled water and by keeping the segment into a humidity chamber for at least 2 hours. Preparation of controls and determination of the normal and abnormal values (or variant)

of Hb was done according to the manufacturer standards and guidelines for the kit. In this programme, Hb Bart's migrates more anodic than Hb F (Fig. 1c).

Polymerase Chain Reaction (PCR) for α-thalassaemia

The PCR analysis was performed on cases that was found to be have Hb Bart's by Hb electrophoreses and on 50 randomly selected samples which were Hb Bart's negative by the Hb electrophoreses. Genomic DNA was extracted from white blood cells (WBC) of the EDTA-anticoagulated peripheral blood samples using Qiagen Blood Mini DNA Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's guidelines. The DNA concentration and purity was determined spectrophotometrically at 260 nm and 280 nm and DNA samples were stored at -30°C before use.

We optimized a gap-polymerase chain reaction (gap-PCR) assay capable of detecting any combination of the six common a-globin gene deletions utilizing the primer sets established by Chong *et al*, that was able to detect the common mutations in our region, -SEA deletion, $-\alpha^{3.7}$ rightward deletion, and $-\alpha^{4.2}$ leftward deletions.[8] The reaction mix was subjected to the following PCR conditions; 96°C for 15 minutes for initial denaturation, followed by 30 cycles of denaturation at 98°C for 45 seconds, annealing at 67°C for 1 minute and 30 seconds and extension at 72°C for 2 minutes and 30 seconds. Amplicons were size fractionated with the standard agarose gel electrophoresis.

The same samples were also subjected to a second phase single tube multiplex amplification refractory mutation system-PCR (ARMS-PCR) assay to screen for non-deletional a-globin gene mutations based on the methods published by Eng *et al*[9].

The standard reaction mix contained 1 mg DNA, 10 pmol of each primer and 1.5 U of Taq DNA polymerase (Qiagen) and was subjected to PCR amplification at 94°C for 12 minutes for initial denaturation, followed by 32 cycles of denaturation at 94°C for 40 seconds, annealing at 62°C for 20 seconds and extension at 72°C for 3 minutes. Amplicons were size fractionated with the standard agarose gel electrophoresis.

Statistics

Bland-Altman methods with one sample t-test was used to compare the data obtained from CE with both, NF and CB programmes to assess the level of agreement between the two measurements and the scatter plot of the differences between the measurements and their means were constructed to determine the agreement.

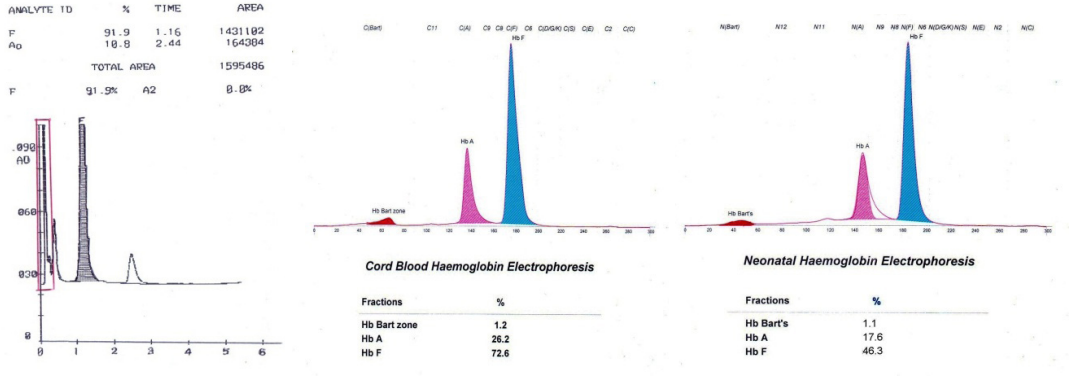
RESULTS

Hb Bart's detection by haemoglobin electrophoresis

32 of 600 samples (5.3%) showed Hb Bart's peak by NF programme, while one additional sample (33/600, 5.5%) was detected to have Hb Bart's by CB programme and HPLC (Table 1, sample 30). 22/33 (66.6%) were males and 11/33 (33.3%) were females. In terms of ethnicity, 19 were Malays (57.5%), 12 Chinese (36.3%) and 1 Vietnamese (3%).

*α-thalass**aemia genotypes*

α-genotyping by single tube multiplex ARMS



(a) **(b)** **(c)**

FIG. 1: Hb Bart's detection by the three methods: a) HPLC (boxed red), b) CE using cord blood programme (whole blood) and c) CE using Neonat Fast kit (DBS)

TABLE 1: Hb Bart's detection by haemoglobin electrophoresis and their α-genotypes

	Sebia Capillarys 2 Neonat Fast Hb programme			Sebia Capillarys 2 Cord Blood programme			HPLC Beta-thalassaemia short programme			α-genotypes
	HbA (%)	HbF (%)	Others (%)	HbA (%)	HbF (%)	Others (%)	HbA (%)	HbF (%)	Others (%)	
1	17.6	46.3	*B: 1.1	26.2	72.6	B:1.2	27.0	76.0	**Π	αα/_ _^{SEA}
2	8.3	42.8	B:2.3/ E:2.4	14.9	79.4	B:1.2	15.0	83.9	Π	αα/_ _^{SEA}
3	12.1	52.4	B:1.3	20.0	79.0	B:1.0	20.3	83.3	Π	αα/_ _^{SEA}
4	8.4	54.6	B:1.3	14.3	82.3	B:3.4	14.2	89.9	Π	αα/_ _^{SEA}
5	15.3	41.9	B:0.9	31.3	67.1	B:1.4/ A2:0.2	28.3	73.1	Π	αα/_ _^{SEA}
6	13.3	47.7	B:1.6	22.2	76.1	B:1.7	23.7	79.8	Π	αα/_ _^{SEA}
7	16.8	51.5	B:1.0	24.1	75.2	B:0.7	25.7	77.6	Π	αα/_ _^{SEA}
8	7.4	61.4	B:1.0	12.9	85.5	B:1.6	13.7	91.2	Π	αα/_ _^{SEA}
9	7.2	56.5	B:0.6	13.3	85.7	B:1.0	14.4	90.5	Π	αα/_ _^{SEA}
10	24.2	36.1	B:0.6	42.7	56.0	B:1.0	41.1	59.6	Π	αα/_ _^{SEA}
11	16.0	53.8	B:1.8	23.2	74.5	B:2.3	23.8	79.7	Π	αα/_ _^{SEA}
12	18.1	48.2	B:1.3/ ***E:2.3	26.8	71.9	B:1.3	28.0	75.2	Π	αα/_ _^{SEA}
13	13.7	56.6	B:1.6	19.8	78.6	B:1.6	19.9	83.9	Π	αα/_ _^{SEA}
14	15.5	55.3	B:1.6	22.6	75.6	B:1.8	22.3	81.0	Π	αα/_ _^{SEA}
15	6.0	59.5	B:2.4	9.9	87.9	B:2.2	10.8	91.9	Π	αα/_ _^{SEA}
16	19.9	47.3	B:0.7	31.8	67.5	B:0.7	30.8	70.0	Π	αα/_ _^{SEA}
17	9.8	58.3	B:2.1	15.4	81.2	B:3.4	15.7	85.9	Π	αα/_ _^{SEA}
18	20.1	47.8	B:1.9	30.8	68.0	B:1.1/ A2:0.1	31.3	71.4	Π	αα/_ _^{SEA}
19	22.0	40.5	B:1.0	38.1	58.9	B:3.0	37.7	64.0	Π	αα/_ _^{SEA}
20	13.3	58.2	B:2.1	8.6	88.7	B:2.7	19.2	84.5	Π	αα/_ _^{SEA}
21	17.3	50.0	B:2.0	26.8	70.9	B:2.3	26.5	76.0	Π	αα/_ _^{SEA}
22	14.7	55.8	B:2.0	21.9	76.6	B:1.5	22.4	81.3	Π	αα/_ _^{SEA}
23	13.3	53.6	B:2.1	24.1	73.8	B:2.1	22.7	80.1	Π	αα/_ _^{SEA}
24	9.6	57.3	B:1.6	15.1	84.0	B:0.9	15.3	89.7	Π	$-\alpha^{3.7}/-\alpha^{3.7}$
25	16.3	44.8	B:0.7	26.9	72.5	B:0.6	25.9	77.4	Π	$-\alpha^{3.7}/-\alpha^{3.7}$
26	12.6	45.3	B: 0.8	20.8	78.5	B:0.7	22.5	80.9	Π	$-\alpha^{3.7}/-\alpha^{3.7}$
27	11.5	50.3	B:1.3/ E:2.3	13.3	82.5	B:0.75/ E:3.5	20.1	78.1	A2: 5.2 Π	$-\alpha^{3.7}/-\alpha^{3.7}$
28	16.2	51.9	B:3.3	25.2	64.8	B:5.0	25.1	78.8	Π	$-\alpha^{3.7}/-\alpha^{3.7}$
29	14.6	56.0	B:3.8	20.8	72.9	B:6.3	21.7	81.9	Π	$-\alpha^{3.7}/-\alpha^{3.7}$
30	8.9	61.4	B:0.0	14.9	84.6	B: 0.7	14.0	88.4	Π	$-\alpha^{3.7}/-\alpha^{3.7}$
31	12.4	59.8	B:0.6	18.9	80.6	B:0.5	17.7	86.0	Π	$\alpha\alpha/\alpha\alpha^{CS}$
32	7.0	57.9	B:0.5	10.7	88.7	B:0.6	10.6	94.8	Π	$\alpha\alpha/\alpha\alpha^{CS}$
33	7.4	52.4	B:4.1/ C:4.6	11.9	73.9	B:7.1/ C:7.1	12.2	78.9	Π	$\alpha\alpha^{CS}/\alpha\alpha^{CS}$
34	10.8	54.6	-	17.5	82.5	-	16.8	88.2	-	$\alpha\alpha/-\alpha^{3.7}$
35	13.7	54.9	-	17.7	82.3	-	21.3	82.5	-	$\alpha\alpha/-\alpha^{3.7}$
36	9.7	57.2	-	16.0	84.0	-	16.8	86.0		$\alpha\alpha/-\alpha^{4.2}$

*B = Hb Bart's, ***E = Hb E, **Π = Hb Bart's peak

TABLE 2: The means of Hb Bart's levels in α-thalassaemia genotypes

α-thalassaemia genotypes	Hb Bart's level by NF programme (%)	Hb Bart's level by CB programme (%)
αα/ -$\alpha^{3.7}$	3.55	5.65
-$\alpha^{3.7}$/-$\alpha^{3.7}$	1.10	0.70
αα/--^{SEA}	1.45	1.60
$\alpha\alpha^{CS}$ /$\alpha\alpha^{CS}$	4.10	7.10
αα/ $\alpha\alpha^{CS}$	0.50	0.55

PCR were done for all Hb Bart's positive samples (33) and 50 negative samples. A total of 36/83 (43.4%) cases were found to carry α-thalassaemia mutations. 33/36 of these cases also showed presence of Hb Bart's by CE and HPLC. However, 3/36 cases from the Hb Bart's negative samples by Hb electrophoresis were also confirmed to be α-thalassaemia carriers by the multiplex ARMS PCR. These cases were; one Chinese male with αα/ -$\alpha^{4.2}$ genotype, one Bajau male and one Murut female, both with αα/ -$\alpha^{3.7}$ genotype (Table 1, cases 34-36). The α-thalassaemia genotypes are presented in Table 1.

All the samples that showed presence of Hb Bart's by the three electrophoreses methods were confirmed to carry α-gene mutation resulting in their specificity of 100%. However, the three samples with one-gene deletion; two with αα/-$\alpha^{3.7}$ genotype and one with αα/-$\alpha^{4.2}$ genotype, did not show Hb Bart's peak by HPLC or CE. In addition, one more sample with one-gene deletion (αα/-$\alpha^{3.7}$) were identified to have Hb Bart's by CE with CB programme (Table 1, case 30). Thus, the sensitivity of HPLC and CE with CB programme was 91.67% for α-thalassaemia detection. Sensitivity of CE NF programme was slightly lower at 88.89%.

Hb Bart's levels

The means of Hb Bart's by the various techniques are shown in Table 2. The means was higher in (αα/ -$\alpha^{3.7}$) than the other groups of thalassaemia ($p < 0.05$). On the other hand, Hb Bart's level was the lowest in the heterozygous Constant Spring cases (αα/ $\alpha\alpha^{CS}$) by both NF and CB programmes.

Although the three methods showed the same specificity (100%), with no significant difference in the sensitivity in the detection of Hb Bart's, the two CE programmes did not show sufficient agreement between the means of Hb Bart's level ($p < 0.05$). Thus, they cannot be used interchangeably.

DISCUSSION

It has long been recognized that the presence of Hb Bart's in the newborn is associated with α-thalassaemia, most commonly due to genetic deletions. All the known α-gene deletions have been reported to produce a variable amount of Hb Bart's in the first few months of life as a result of imbalance in the production of α and β globins. The level of Hb Bart's in the blood is reported to correlate with the number of deleted genes.[10] Hence, in neonates, the diagnosis of α-thalassaemia could be ascertained by Hb Bart's detection.

We found 5.5% of our samples to have Hb Bart's peak, indicating presence of α-thalassaemia as confirmed by the molecular analysis. This prevalence is comparable to the previous studies done in Malaysia.[11,12] Most of the positive samples were of Malay ethnicity (57.5%), followed by Chinese (36.3%).

All of the samples detected to have Hb Bart's were confirmed to carry α-thalassaemia mutation/s, resulting in specificity of 100% of these methods. However, both HLPC and CE with CB programme failed to detect the Hb Bart's only in three of 36 of confirmed α-thalassaemia cases. This could be explained by the fact that some newborns with α-thalassaemia trait do not always have detectable levels of Hb Bart's at birth.[3]

All Hb Bart's positive cases as detected by NF programme were in agreement with the HPLC system. In addition, there was one more case with heterozygous 3.7 deletion, in which, the amount of Hb Bart's was unquantifiable by NF programme. Therefore, CB programme using a fresh cord blood showed superiority in Hb Bart's detection and quantification over NF programme.

The measurement of Hb Bart's could be less straight-forward with the NF programme due to the inevitable degradation effect of the extracted dried blood, which leads to a higher baseline that interferes with the integrity of the eluted

Hb fractions. The degraded products appear more prominent at the middle of the histogram, where both the HbF and the HbA fractions are eluted on top of an elevated base line. However, Hb Bart's does not overlap with the degradation products and the fraction is well visible, because Hb Bart's was eluted at the beginning of the histogram. Therefore, there was a small peak present in zone N13 (20-40) for Hb Bart's only (Fig. 1c).

Further investigations has found that there are three slightly different deletions for $-\alpha^{3.7}$, which are now designated $-\alpha^{3.7I}$, $-\alpha^{3.7II}$ and $-\alpha^{3.7III}$. While $-\alpha^{3.7I}$ is common in many ethnic groups, the $-\alpha^{3.7II}$ was confined to India and Nepal, whereas $-\alpha^{3.7III}$ to Oceania.[13] However to our knowledge, in the available literature, there is no study that showed the difference of Hb Bart's levels in these three different deletions. We also did not have the facilities to differentiate the three deletional subtypes at the molecular level.

By the three methods used in this study, most of the common variants in homozygous and heterozygous states of α-gene deletion as well as Hb CS were clearly recognized by the presence of Hb Bart's peak. However, there was no direct proportional relationship between the number of gene deletion and Hb Bart's level in this study as there were marked overlaps in Hb Bart's levels between the different deletions. Harteveld and Higgs also reported that not all cases of α-thalassaemia carriers demonstrated Hb Bart's at birth and its level could not differentiate the different α-genotypes.[14]

α-globin chain variant ($\alpha\alpha^{CS}$) producing Hb Constant Spring (Hb CS) was also detected in homo and heterozygous forms; only in the homozygous form the analyzer clearly demonstrated peaks of Hb CS (4.1%), with Hb Bart's (4.5%) (Table 1). In contrast, only Hb Bart's peak was observed in heterozygous form. None of the heterozygous CS cases showed Hb CS peak by the three methods as Hb CS is known for its unstable nature. Thus, the detection of Hb Bart's is more sensitive than Hb CS to detect this condition in the neonatal period.[15]

The levels of Hb Bart's detected by CB programme and by NF programme were different in most of the cases; the highest level of Hb Bart's detected by the CB programme was 7.1% while the NF programme gave a result of 4.1% for the same case. However, the lowest was 0.5% with both kits for the same case. On the contrary, in some other cases, the Hb Bart's detected by NF programme was higher than by CB for the same cases. In general, the means of Hb Bart's level of each genotype detected by the CE with NF programme were lower than the means given by the CB programme. Statistically, the two kits did not show sufficient agreement to be used interchangeably. Of note, there were none on the 47 samples randomly analyzed for α-thalassaemia by molecular analysis demonstrated Hb Bart's by all three methods.

It is not uncommon to find other Hb variants in compound heterozygous state with α-thalassaemia. In one study conducted by Supan Fucharoen on Capillarys 2 Hemoglobin testing system, 122 out of 226 newborns were found to have α-thalassaemias with 17 different genotypes, Hb E was found to be 2.6–6.2% in heterozygous or homozygous α+-thalassaemia and heterozygous α^0-thalassaemia.[6] In our study, there were two babies with $--^{SEA}$ deletion and one baby with $-\alpha^{3.7}/-\alpha^{3.7}$ who had concomitant Hb E. Their Hb Bart's levels were similar or even less as compared to other babies with $--^{SEA}$ or $-\alpha^{3.7}/-\alpha^{3.7}$ without any concomitant Hb variants (Table 1).

Neonatal α-thalassaemia screening is quite a difficult undertaking by the molecular technique. We are using a single tube multiplex PCR technique to detect 7 deletions and 4 α gene mutations in our laboratory. However this technique could only identify the common deletions and mutations in Malaysia. It is too laborious, slow and in the long run, expensive to cover a large number of deletions and mutations, with the cost of RM400 (USD100) for just one case. In comparison, the cost of analyzing each sample by CE is only RM 22. Therefore, cord blood analysis by CE for Hb Bart's can potentially screen α-gene mutations, including the rare or even a novel mutation that leads to Hb Bart's production in the neonatal period. However, positive Hb Bart's detection by CE should always be considered provisional. Presumably affected subjects and their parents should always be confirmed at molecular level.

CE is a highly automated analyzer where up to seven samples can be loaded onto its eight well-plates and a complete 'walk away' for the operator with very short analysis time (48 samples per hour). The only drawback lies only in the DBS sample preparation step which is still slightly laborious. After drying of the blood spot on Guthrie card for three to four hours at room temperature, the incubation time of the haemolysate preparation in the humidity chamber requires up to two hours for new samples and even more for the aged ones.

DBS samples use only a few drops of blood from the cord blood samples collected for G6PD screening. This offers a practical and a significant cost saving option in terms of logistics of sample collection, transport to reference laboratory, sample processing and storage, especially for samples from remote hospitals. In conclusion, CE using either fresh cord blood specimens or HPLC were able to detect α-thalassaemia carrier with an acceptable sensitivity especially in cases involving more than two α-gene deletions or mutations. The lowest Hb Bart's level by CE that could detect α-thalassaemia in this study was 0.5%.

REFERENCES

1. Giambona A, Passarello C, Renda D, Maggio A. The significance of the hemoglobin A_2 value in screening for hemoglobinopathies. Clin Biochem. 2009; 42: 1786-96.
2. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem. 2000; 46: 1284-90.
3. Galanello R, Cao A. Alpha-thalassemia. Genet Med. 2011; 13: 83-8.
4. Mantikou E, Arkesteijn SG, Beckhoven van JM, Kerkhoffs JL, Harteveld CL, Giordano PC. A brief review on newborn screening methods for hemoglobinopathies and preliminary results selecting beta thalassemia carriers at birth by quantitative estimation of the HbA fraction. Clin Biochem. 2009; 42: 1780-5.
5. 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.
6. Srivorakun H, Fucharoen G, Changtrakul Y, Komwilaisak P, Fucharoen S. Thalassemia and hemoglobinopathies in Southeast Asian newborns: diagnostic assessment using capillary electrophoresis system. Clin Biochem. 2011; 44: 406-11.
7. Pornprasert S, Kasemrad C, Sukunthamala K. Diagnosis of thalassemia on dried blood spot samples by high performance liquid chromatography. Hemoglobin. 2010; 34: 486-94.
8. Chong SS, Boehm CD, Higgs DR, Cutting GR. Single-tube multiplex-PCR screen for common deletional determinants of α-thalassemia. Blood. 2000; 95: 360-2.
9. Eng B, Patterson M, Walker L, Chui DH, Waye JS. Detection of severe nondeletional alpha-thalassemia mutations using a single-tube multiplex ARMS assay. Genet Test. 2001; 5: 327-9.
10. Bain BJ, editor. Haemoglobinopathy Diagnosis. 2nd ed. Oxford, UK: Blackwell Publishing Ltd; 2006.
11. Azma RZ, Ainoon O, Hafiza A, *et al*. Molecular characteristic of alpha thalassaemia among patients diagnosed in UKM Medical Centre. Malays J Pathol. 2014; 36: 27-32.
12. Ahmad R, Saleem M, Aloysious NS, Yelumalai P, Mohamed N, Hassan S. Distribution of alpha thalassaemia gene variants in diverse ethnic populations in Malaysia: data from the Institute for Medical Research. Int J Mol Sci. 2013; 14: 18599-614.
13. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001; 79: 704-12.
14. Harteveld CL, Higgs DR. α-thalassaemia. Orphanet J Rare Dis. 2010; 5: 13.
15. Tang HS, Zhou JY, Xie XM, Li DZ. Newborn screening for α-thalassaemia by a capillary electrophoresis method. J Med Screen. 2012; 19: 159.