ORIGINAL ARTICLE

Significance of Zone 2 Peak on Capillary Electrophoresis (ce) for the Detection of Haemoglobin Constant Spring (Hb CS) among General Population in Kelantan

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ABSTRACT

Introduction: **Haemoglobin Constant Spring (Hb CS) is a point mutational defect of α thalassaemia at the termination codon and leads to α chain extension and reduced mRNA stability. Hb CS is unstable haemoglobin and is low level in peripheral blood for detection. However, the capillary electrophoresis (CE) is able to separate and quantitate Hb CS. The aim of the present study was to determine the significance presence of Zone 2 peak on capillary electrophoresis (CE) in diagnosing Hb CS among general population in Kelantan.**

Materials and Methods: **One hundred and thirty-seven patients' sample were screened by CE and proceeded with molecular analysis. In the CE electrophoregram, Hb CS and Hb Paks was detected as having a peak at Zone 2.**

Results: **118 samples out of 137 samples showed a positive peak in Zone 2 of CE and were also confirmed for termination codon mutation for Hb CS by molecular analysis. 19 samples showed negative results for termination codon mutation for Hb CS and termination codon mutation for Hb Paks**

Conclusions: **Hb CS quantification can be predicted by CE method based on peak at Zone 2 if the molecular technology is not available.**

KEY WORDS

haemoglobin constant spring (hb cs), capillary electrophoresis (ce), zone 2

INTRODUCTION

Haemoglobin (Hb) Constant Spring (CS) is a non-deletional alpha (α) thalassaemia on α2 genes as a result of impaired RNA translation consequent on a termination codon mutation leading to an elongated messenger ribonucleic acid (mRNA) and α globin chain. This variant contains point mutation at the termination codon, in which the defect is (TAA > CAA) and it is associated with reduced mRNA stability and elongation of α chain. This point mutation leads to α chain extension up to another 31 amino acid residues[1]. The abnormal Hb in CS leads to unstable mRNA and cause decrease in the rate of α globin chain synthesis.

Hb CS is one of the most prevalent non deletional α thalassaemia in Southeast Asia. Hb CS is the most common non deletional thalassaemia compared to the other two types of mutational α thalassaemia identified which are Hb Adana and Hb Quong Sze. In Malays and Sabahans, it is the third common α thalassaemia determinants. Orang Asli aborigines recorded the highest incidence of Hb CS with the incidence of 11.5% meanwhile the Malaysian Chinese recorded the lowest incidence of 0.7%[2].

The mRNA of alpha Constant Spring (α^{CS}) only accounts for less than 1% of protein output of a normal α2 gene but it is very unstable compared to normal α mRNA. In terms of pathophysiology, the synthesis of even small amounts of elongated α^{CS} results in more severe anaemia with harmful effects on cellular and membrane properties of red blood cells with Hb CS[3]. Previous study found that the Hb CS-containing red blood cells were distinctly overhydrated had occurred early in erythroid maturation and fully expressed at the reticulocyte stage. Membrane rigidity and membrane mechanical stability of Hb CS-containing red blood cells were increased when compared with Hb H and α-thalassemia-1 trait red blood cells caused by combination of the deleterious effects induced by membrane oxidized by α^{CS} and β globin chains[4].

Capillary electrophoresis (CE) is widely used in clinical laboratories

Table 1: Demographic of Hb CS samples in the study

		Number of samples (n = 137)	Percentage (%)
Ethnic group	Malay	132	96.4
	Siamese	5	2.9
	Chinese	1	0.7
Sex	Male	28	20.4
	Female	109	79.6

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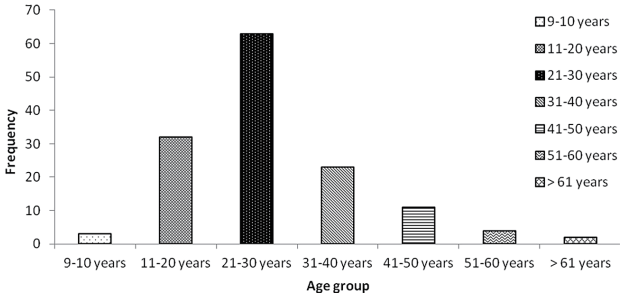

Figure 1: Distribution of samples by age group.

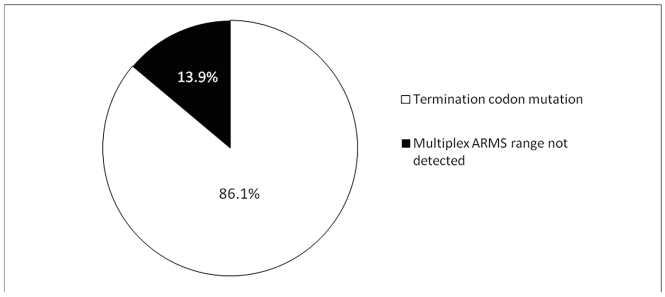

Figure 2: Percentages of samples that showed positive peak in Zone 2 in relation to molecular results.

as an analytical separation technique to separate and quantitate normal haemoglobin (Hb A, A□ and F) as well as detect the major haemoglobin variants such as Hb C, E, S and D (Sebia, 2013). CE separates protein at a high voltage in order to detect haemoglobin at the cathodic end of the capillary using an absorbance wavelength of 415 nm resulted in electrophoregrams for different pattern of abnormalities[5]. Different haemoglobin variants are identified in zones Z1 to Z15. CE system was approved by the USA Food and Drug Administration in 2007 for the evaluation of haemoglobinopathies[6]. For Hb CS, it is identified with the peak in Zone 2 of CE.

Previous study reported that CE testing system has the ability in assisting presumptive diagnosis of Hb variants including five α chain and nine β chain variants commonly found in Southeast Asia such as Hb CS and Hb Paksé[7]. Although these two haemoglobin variants were unstable and their level in peripheral blood was small and difficult to be detected especially in heterozygote group, the CE still could identify them as Hb CS has higher prevalence than Hb Paksé, it is recommended to consider a small peak at Zone 2 as Hb CS[8]. CE had an advantage over HPLC for Hb CS detection[9]. Therefore, the present study aimed to study the significance of Zone 2 peak on CE for Hb CS.

MATERIALS AND METHODS

The blood samples were collected in anticoagulated with ethylenediamine tetraacetic acid (EDTA) obtained from patients that had been suspected to have thalassaemia or haemoglobinopathy from the clinical diagnosis or after getting full blood picture reports from Hospital Raja Perempuan Zainab 2 (HRPZ 2). Ethical approval was obtained from the ethics committee in National Medical Research Registry (NMRR) (NMRR-15-724-25404 IIR) and Hospital Universiti Sains Malaysia (HUSM) (USM/JEPem/15050169). Samples were subjected to full blood count (FBC) (Sysmex XN 3000 analyzer, Sysmex Corporation, USA) and full blood picture.

Prior to CE separation, the sample was diluted in haemolysing solution. Samples were separated and quantitated using CAPILLARYS2 Flex Piercing System (Sebia, PN 1227, France) according to the manufacturer's instructions. The detection of haemoglobin was conducted by measuring absorbance at 415 nm. Presumptive identification of the haemoglobin types located in various zones from Zone 1 to Zone 15 was recorded from electrophoregram. Position of Hb CS was identified in the pertinent zone, Zone 2. Molecular analysis was performed to confirm point mutation at termination codon for Hb CS (TAA→ CAA). Descriptive frequency was used for other variables such as age, sex, race, range of CS level and percentages of positive test for molecular in relation to CS level.

RESULTS

In this study, a total of 137 samples were Malays, Siamese and Chinese (Table 1). Majority of the samples were in 21-30 years of age (Figure 1). 118 samples out of 137 samples showed positive peak in Zone 2 of CE, positive for termination codon mutation for Hb CS (TAA→CAA). 19 samples showed negative results for termination codon mutation for Hb CS and termination codon mutation for Hb Paksé (Figure 2). Eight samples showed other types of α thalassaemia inheritance and one of the cases was Haemoglobin E (Table 2). Seven samples showed normal results for molecular analysis for deletional and non-deletional α mutation. The samples were not tested for molecular analysis of β thalassaemia.

DISCUSSION

This study was conducted in Kelantan, which is situated in the northeast of Peninsular Malaysia. The population of Kelantan in 2014 was 1.4 million people comprising of 95% Malay, 3% Thai (Siamese), 1.9% Chinese and 0.1% others[10]. Samples were taken from HRPZ 2 in which it is the tertiary and referral hospital for Kelantan state. Therefore, the samples can represent true population of Kelantan.

Most of the samples that showed positive peak on Zone 2 CE were from Malay patients, followed by Siamese and only one sample of Chinese patient. Siamese population is also common in Kelantan as it is nearby the border of Thailand. About 80% of the samples came from female as the screening for thalassemia and haemoglobinopathy in Malaysia, targetting the antenatal group as well as pre-marital screening.

It also reflected by the distribution of samples according to age group, in which 46% of the samples were from age 21-30 years old. This showed that most of the samples were from reproductive age group who will have a regular antenatal check up and easily will be picked up for doing haemoglobin analysis if they had hypochromic picture with or without anaemia (MCH < 27 pg)[11].

In the ideal situation, the thalassaemia screening should be done in an adolescent or pre-marital age in order to reduce the birth of new cases of thalassaemia and haemoglobinopathy. Despite that, other timing or age group suitable for thalassaemia screening are newborn, preconceptional and antenatal age group[12].

CE gives a peak at the Zone 2 for Hb CS. However, Hb Paks also shares the same peak and clinical presentation with Hb CS. However, the prevalence of this type of variant has not been described in Malaysia previously. In Thailand and Southern China, the majority of non deletional type of α thalassaemia mutation is Hb CS[13]. Therefore, if suspected to have a Zone 2 peak on CE and non deletional α thalassaemia, usually the screening will be focussed to determine the presence of Hb CS.

The possible causes of a false positive peak on Zone 2 of CE are possible due to plasmatic proteins from the sample. For instance, the sample was taken from a patient with low haemoglobin, with decreased red blood cells per plasma ratio[5]. Another possible reason for zone 2 peak on CE is might due to presence of other Hb variants that share the same zone with Hb CS.

This study did not evaluate the sensitivity of detection of Hb CS using CE compared to HPLC. A CE method has proven sensitive to detect Hb CS trait compared to HPLC as 100%[9]. Using automated HPLC in identifying Hb CS in a screening programme is quite not helpful due to low sensitivity of HPLC[14]. Therefore, CE can be used to predict Hb CS if the molecular test is not available.

CONCLUSION

In this study, most of the samples showed a positive peak in Zone 2 of CE and confirmed for termination codon Hb CS mutation. Therefore, it showed that presence of Zone 2 peak in CE had a significant value in determining Hb CS. CE methods is a good tool to screen for Hb CS.

CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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