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Original Research Article

A Role of Capillary Zone Electrophoresis in Hemoglobinopathy Diagnosis

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Abstract:

Diabetes and hemoglobinopathy can co-exist, but the extent of diagnosis of such co-existence may depend on choosing the appropriate diagnostic methodology. This case report emphasizes the importance of such methods like HPLC and capillary electrophoresis in diagnosing these disorders. Haemoglobin Constant Spring (Hb CS) is one of the most common non-deletional types of alpha (α) thalassaemia in Southeast Asia region. The nature of this abnormal globin gene is that it is unstable, labile and is present in minute amount in the peripheral blood. Thus, this may lead to underdiagnosis of the disease. This study was conducted to determine the proportion of Hb CS among the Kelantan population and to compare range of peak value in Zone 2 CE findings for 3 groups of Hb CS (heterozygous, homozygous, and compound heterozygous) and their haematological parameters. The study aimed to look at the findings of HPLC in relation to CE results in detecting Hb CS.

Keywords: HPLC, Hb D, Hemoglobin disorders, CZE.

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Introduction

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.1 Presently, hemoglobin A1c (HbA1c) holds the monopoly in monitoring the glycemic control in patients with diabetes mellitus.2 High-performance liquid chromatography (HPLC) has been recognized as one of the reliable methodologies for HbA1c measurement. It has also been extensively used for screening hemoglobinopathies. Hence, efficient use of HPLC may serve as an effective tool for dual diagnosis of diabetes and hemoglobinopathy. Alpha (α) thalassemia is one of the most common inherited haemoglobin disorders across the world, characterised by the absence or reduced α-globin gene due to mutation or deletion of α globin gene (Galanello and Cao, 2011). Southeast Asian countries including Malaysia have higher prevalence of α-thalassemia compared to β-thalassemia carrier which is 15.8% and 4.5% respectively. Thus, it is a public health concern in Malaysia (Wee *et al.*, 2005). Haemoglobin (Hb) Constant Spring is one of the non-deletional α-thalassemia involving the α2 gene as the result of impaired RNA translation due to termination codon point mutation cause the defect (TAA>CAA) and resulted in the elongation of the α chain up to another 31 amino acid residues (Liao *et al.*, 2010). The natural features of haemoglobin CS lead to the decrease in the rate of normal α- globin synthesis due to its mRNA instability. Generally, heterozygote Hb CS has normal clinical and haematological features. On the other hand, homozygotes Hb CS may present as thalassemia intermediate with mild anaemia, jaundice, and hepatosplenomegaly. However, the interaction of CS gene with deletional type of alpha-thalassemia is the main cause of non-deletional Hb H (β4) (--/$\alpha^{cs}\alpha$). This non- deletional α thalassemia is more severe than deletional α-thalassemia, where some patients became transfusion-dependent (Pornprasert *et al.*, 2016) (Liao *et al.*, 2010). There are several methods of haemoglobin analysis available worldwide. Capillary electrophoresis (CE) and High-Performance Liquid Chromatography (HPLC) are among the widely used method for the screening of abnormal haemoglobin. CE will give a peak at the Zone 2 for Hb CS. Another common variant that also shares the same peak is, Hb Pakse (Singsanan *et al.*, 2007). In HPLC findings, Hb CS gives a very small peak at the C window with the retention time of 4.90 - 5.30 minute (Greene *et al.*, 2012). The aim of this study is to determine the significant of Zone 2 peak on CE in diagnosing Hb CS and its relationship with HPLC findings. Hb CS is often goes undiagnosed due to almost normal red cells indices and is present at a low level in peripheral blood. Currently, the gold standard for diagnosis is still based on molecular

results which is costly and tedious. In Malaysia, only several centres offer these molecular tests. Most of the health centres in Malaysia are still using HPLC as the screening tool and certain centres started to use CE as the main method in detecting haemoglobinopathies.

Materials and Methods

This was a cross-sectional study involving secondary data collection from 378 samples which showed peak value on Zone 2 of CE. The haematological parameters of red cells were analysed using Sysmex XN 3000 automated blood cell analyser, Hb analysis was performed using automated CE system (CAPILLARYS2 Flex-Piercing System Sebia), HPLC Biorad variant II, DNA analysed using multiplex polymerase chain reaction (PCR) and multiplex Amplification refractory mutation system (ARMS) to detect both deletional and non-deletional α-thalassaemia.

Results

376 samples (99.5%) with presence of peak value on Zone 2 of CE were confirmed to have termination codon CS mutation. Heterozygous Hb CS is the most common type of Hb CS detected in 344 samples (91.5%), followed by compound heterozygous Hb CS which was 31 samples (8.2%) and only 1 sample (0.3%) of homozygous Hb CS. The mean ± SD of peak value in Zone 2 of heterozygous Hb CS and compound heterozygous Hb CS were 0.61 ± 0.13 and 0.77 ± 0.34 respectively. The only sample of homozygous Hb CS showed the value of 4.9% of peak value in Zone 2 of CE. The significant differences of haematological parameters between heterozygous and compound heterozygous Hb CS were observed in haemoglobin level, MCV, MCH and MCHC. This study showed there was a good linear correlation between peak in C-window on HPLC and peak value in Zone 2 of CE in detecting Hb CS, r=0.73.

Discussion

Hemoglobinopathies are a group of disorders characterized by qualitative or quantitative abnormality of hemoglobin. Approximately 7% of the world's population carries an Hb variant, making hemoglobinopathies one of the most common monogenic diseases. Based on the clinical significance, these variants can be classified as clinically silent variants and clinically manifesting variants. The clinically silent variants are relatively underdiagnosed when compared to the manifesting variants, since there are no clinical manifestations to support the laboratory diagnosis. Hence most of these variants are diagnosed through incidental findings in a clinical laboratory. One of the common approaches towards the incidental discovery of such variants is through testing of HbA1c by HPLC. Moreover, HPLC is one of the commonly used methods across India for HbA1c measurement. In HPLC, HbA1c is measured as a fraction of total hemoglobin. Hemoglobin is separated into several fractions, which appear as peaks in the chromatogram with definite pattern. Apart from HbA1c measurement, HPLC gives value added information on presence of abnormal hemoglobins in a patient. In the present case, all biochemical and hematological investigations were within the reference limit except the HPLC chromatogram (A2F mode), which showed the presence of an abnormal peak (40.9%) at 2.71 minutes. This aroused a high index of suspicion of clinically silent hemoglobin (Fig. 1). As per the British Society of Hematology guidelines (Significant haemoglobinopathies: guidelines for screening and diagnosis), our laboratory has adopted a practice of screening hemoglobinopathies through two methods namely HPLC and CZE.[3] A presumptive diagnosis of hemoglobinopathy is done based on correlation between these two methods.

In the current case, the capillary electrophoretogram showed the presence of an abnormal peak (43.2%) in the Hb D zone, suggestive of the presence of hemoglobin D. In HPLC, hemoglobin D appears at 3.9 minutes, but this did not correlate with the retention time of the abnormal hemoglobin noted in the present patient (2.71 minutes, Fig. 2). There was a diagnostic mismatch between HPLC and capillary electrophoresis. Hence, probing was done to check for the probability of abnormal hemoglobin presenting with this pattern (at Hb D zone in capillary and at 2.71 minutes in HPLC). A review of literature indicated Hb D Iran as the hemoglobin fraction that closely matches with the present case.4

Hb D Iran is prevalent in north western parts of India, Pakistan and Iran.5 There is no convincing evidence on prevalence of Hb D Iran in Southern parts of India. Hence the variant that had been identified in the current patient could be Hb D Iran or any other variants resembling Hb D Iran. The confirmative diagnosis can be arrived only through molecular analysis and this was not performed due to limited resources in the hospital.

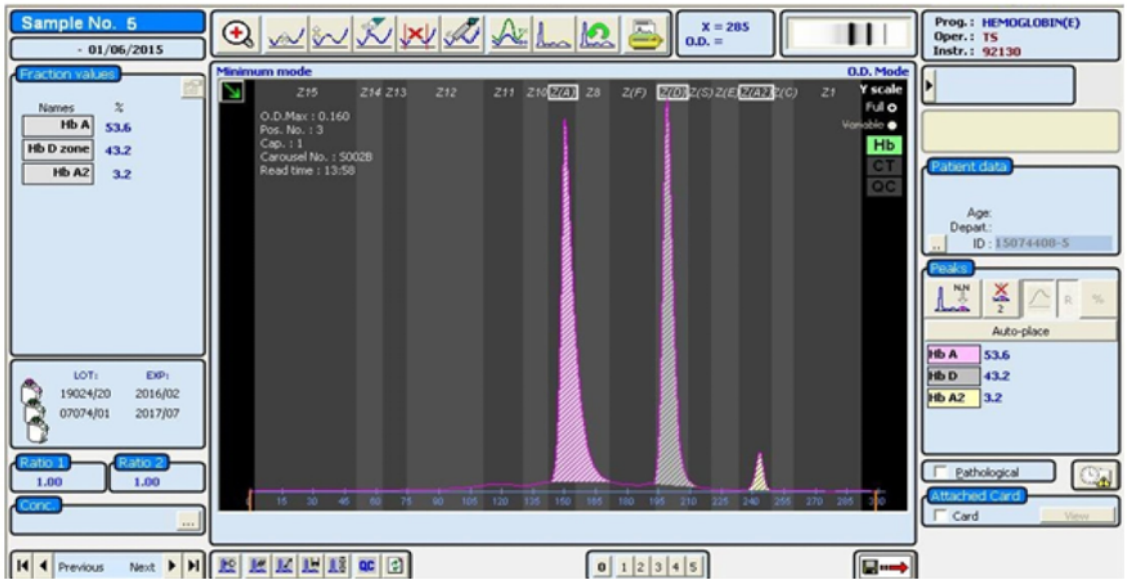

Figure 1: Hemoglobin in HPLC

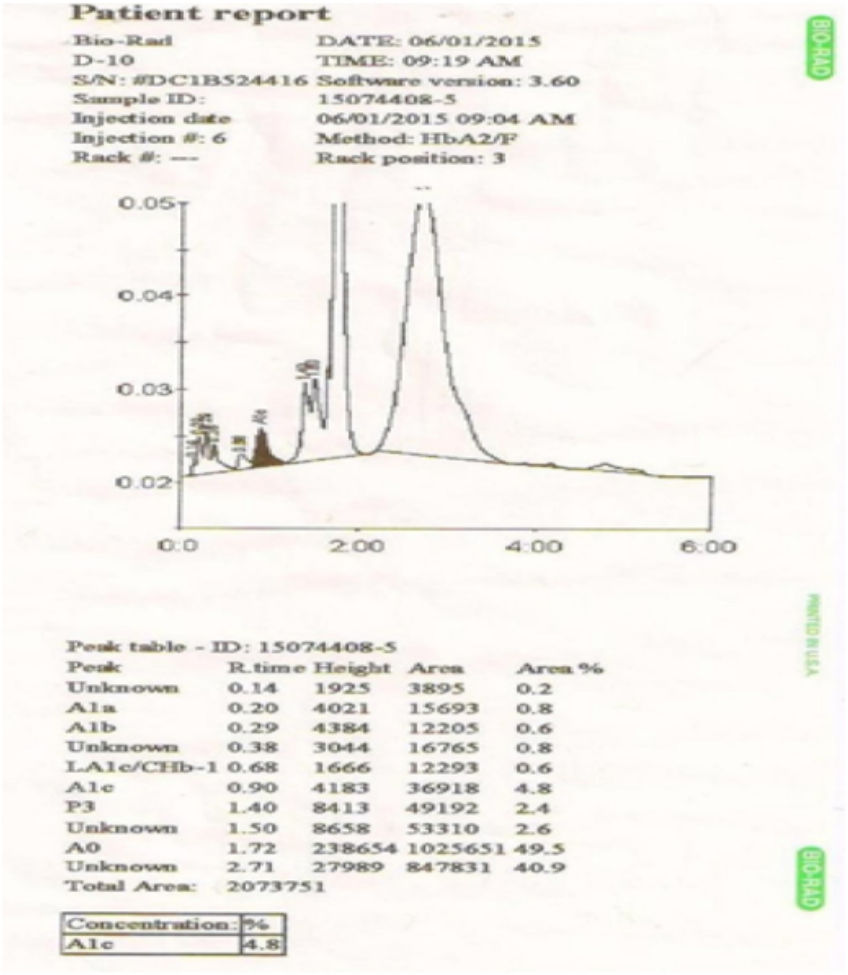

Figure 2: Hemoglobin in capillary electrophoresis

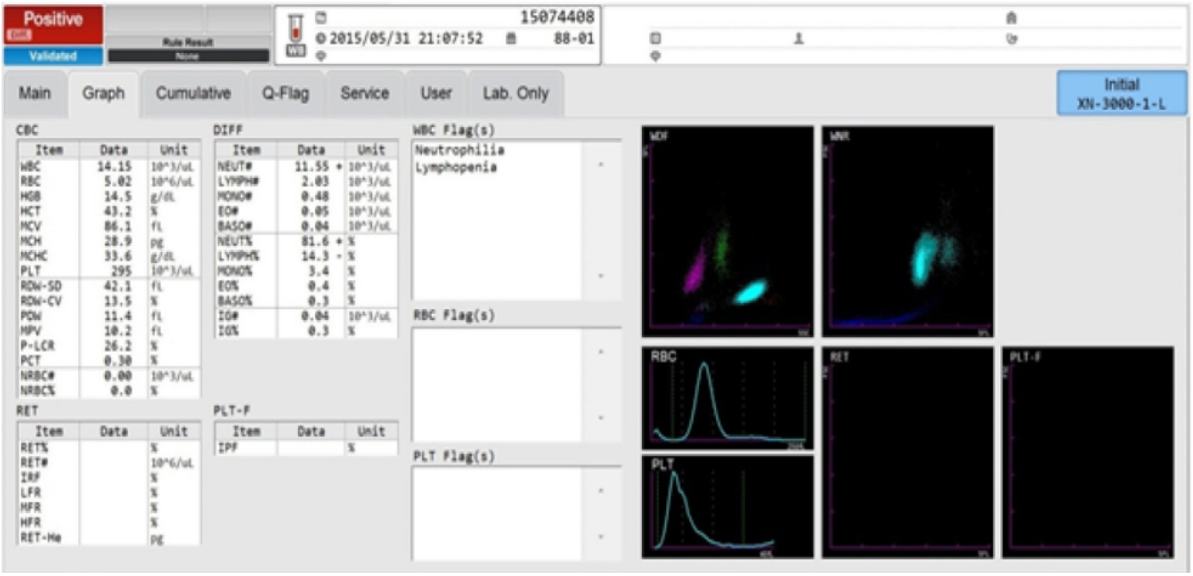

Figure 3: Hemoglobin Complete blood count

Conclusion

This case report may assist in creating awareness in laboratories regarding the presence of abnormal hemoglobins (some of them are clinically silent) in local population. Hence, it is necessary for the laboratorian to have a basic knowledge on understanding and interpreting the chromatograms and electrophoretograms, and this guideline should be implemented in the laboratory practice. To conclude, hemoglobinopathies are preventable, though not treatable, for which implementation of an appropriate diagnostic method with in-depth understanding is required. In addition, to prevent the transmission of these disorders, proper genetic counselling of family members is warranted

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