SHORT COMMUNICATION

Comparison between High Performance Liquid Chromatography and Capillary Electrophoresis in detecting Haemoglobin Constant Spring

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Abstract:

Two popular methods - High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE) have been compared for diagnosis of Haemoglobin Constant Spring (HbCS). The performance of the methods was evaluated in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). A total of thirty-two results were collected at HUKM which consisted of results derived from HPLC, CE and ARMS-PCR from December 2012 until December 2017. Results obtained showed CE demonstrated higher sensitivity, specificity, PPV and NPV compared to HPLC in the detection of HbCS.

Keywords: Capillary Electrophoresis, Haemoglobin Constant Spring (HbCS), High Performance Liquid Chromatography

1. INTRODUCTION

Haemoglobin Constant Spring (HbCS) is an abnormal haemoglobin characterized by elongated α-globin chain resulting from mutation of the termination codon in the α2-globin gene (TAA > CAA). The mutation leads to reduction in α chain synthesis (1% of normal). HbCS is the most common non-deletional α-thalassemic mutation in the South East Asia population. Although HbCS is found in China and South East Asia, it has been sporadically reported in the Mediterranean and Middle East regions. In Malaysia, HbCS has been found in Malay (2.24%), Chinese (0.66%) and Indian (0.16%) populations [1]. The simple heterozygous form of HbCS is minimally anemic while patient with homozygous HbCS or combined heterozygous with other deletional α-thalassemia may develop more severe clinical features [2]. The initial step for screening of HbCS is determination of red cell indices which are mean corpuscular volume (MCV) and mean cell haemoglobin (MCH) [3]. Heterozygous HbCS have normal MCV and slightly low MCH but homozygous HbCS have low values for both MCV and MCH. In terms of RBC morphology, it will show marked anisocytosis, hypochromia and basophilic stippling. In Malaysia, further diagnosis of HbSC requires hemoglobin analysis which uses High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE) methods [4]. Both are the most common and important tool for detecting thalassemia and haemoglobin variants. DNA analysis is used as a validation method in detecting the variants of haemoglobin associated disorders. One of the techniques used is the multiplex amplified refractory mutation system (ARMS) polymerase chain reaction (PCR).

Currently, HPLC and CE are the most popular techniques used in the diagnosis of HbCS. However, CE is not available in most laboratories in Malaysia as compared to HPLC. This is due to the fact that CE is a more advanced technique as compared to HPLC in detecting and quantifying haemoglobin variants and as such is more expensive. Unfortunately, at times, screening for HbCS via HPLC and CE gave different results. Hence the current study was proposed to compare the performance between HPLC and CE in diagnosing HbCS.

2. MATERIALS AND METHODS

Ethical approval from the Research Ethical Committee of UiTM and Hospital Universiti Kebangsaan Malaysia (HUKM) were obtained for this retrospective study. Cases for analysis were selected from patients' data collected from 2012 until 2017 at the Department of Pathology, HUKM, Cheras, Kuala Lumpur.

Results from 32 patients which consisted of positive or negative HbCS cases diagnosed with the three methods; HPLC, CE and ARMS-PCR were analysed. For this study,

results obtained from HPLC and CE were compared to that derived from ARMS-PCR. Data were processed and analyzed by the Statistical Package for Social Science (SPSS) program version 18.0 software. Sensitivity and specificity test were calculated by performing column cross-tabulation while Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated by performing row cross-tabulation.

3. RESULTS AND DISCUSSION

Screening test for this abnormal haemoglobin among Malaysian population is important as earlier study has reported that this inherited disease is the most prevalent non-deletional α-thalassemia in the South East Asia population [5].

ARMS-PCR is one of the many DNA analysis techniques available and commonly used to study haemoglobin variants. It is a more reliable method to be used in the detection of HbCS hence results from ARMS-PCR acted as the bench-mark in the present study. Table 1 summarizes the results of the thirty-two patients analysed in this study.

Table 1: Summarization of results from HPLC, CE and ARMS-PCR.

Result	Number of patients detected using different methods of detection		
	ARMS PCR	HPLC	CE
Positive HbCS	29	4	10
Negative HbCS	3	28	22
Total	32	32	32

Looking at the distribution of results obtained from the three techniques, it was shown that more than half of the samples were detected as negative during screening of this abnormal haemoglobin using HPLC and CE. Based on results obtained using HPLC, only four of the thirty-two patients (12%) were detected as positive for HbCS while the other twenty-eight patients (88%) were negative for HbCS. As for the results of patients diagnosed using CE, ten of the patients (31%) were positive for HbCS while the remaining twenty-two patients (69%) were diagnosed as negative. Alarmingly, ARMS-PCR managed to diagnose twenty-nine out of the thirty-two patients (91%) as positive while only three patients (9%) were negative for HbCS. However, this finding was not surprising as according to Singsanan et al. [6] and Ne et al. [7], HbCS is often missed by routine laboratory screening test, especially if it is in the heterozygote form. This is because this abnormal haemoglobin is unstable and is presented at a low level in peripheral blood. Since there were several discrepancies shown in the results obtained from HPLC and CE, sensitivity and specificity analysis were performed.

Sensitivity refers to the ability of the test to correctly identify patients with disease while specificity refers to the ability of the test to correctly identify patients without the disease. In the present study, both tests used results of ARMS-PCR as their standard.

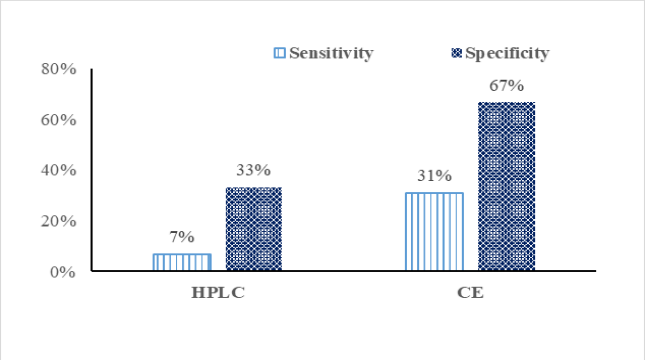

Figure 1: Comparison between sensitivity and specificity of HPLC and CE.

Based on the result shown in Figure 1, sensitivity and specificity value of HPLC were 6.9% and 33.3%, respectively. The sensitivity result showed that only 6.9% out of the thirty-two patients were correctly identified by HPLC as having HbCS. On the other hand, the specificity result demonstrated that one patient was correctly identified by HPLC as not having this abnormal haemoglobin. The sensitivity and specificity of HPLC obtained in the present study was much lower than that reported by earlier researchers. In a previous study, the sensitivity and specificity of HPLC were reported as 93.38% and 99.80%, respectively [8]. As for CE, the sensitivity and specificity value were 31.0 % and 66.7%, respectively.

In the current study, the sensitivity value of HPLC and CE were much lower than their specificity values. The result reflected that there were high amount of false negative results of HbCS from both HPLC and CE. According to Singsanan et al. [6] and Ne et al. [7], the false negative results was probably caused by the small quantity and the lability of HbCS in peripheral blood. Lability of HbCS might be due to the degradation of αCS mRNA in the cytoplasm. Part of the 30 non-coding region of the αCS mRNA is destabilized over the usual termination codon during translation process. Thus, this complication rendered the mRNA susceptible to nucleus attack [8]. When the sensitivity and specificity value of HPLC were compared to CE, it was shown that CE have higher sensitivity and specificity values. Therefore, the results reflected that CE was more sensitive and specific compared to HPLC. This finding is consistent with earlier study which suggested that CE was suitable for Hb CS trait routine screening as it could quantify a HbCS level as low as 0.1% [9].

Positive predictive value (PPV) refers to the probability that subjects which are truly positive for the disease will have a positive result while negative predictive value (NPV) is the probability that subjects which are truly negative for the disease will be negative in the screening test. In this study, PPV was determined to evaluate the probability of positive HbCS patients having positive result when detected by HPLC or CE when compared with confirmative ARMS-PCR analysis. On other hand, NPV was obtained to determine the probability of negative HbCS patients having negative result via HPLC and CE when compared with molecular analysis.

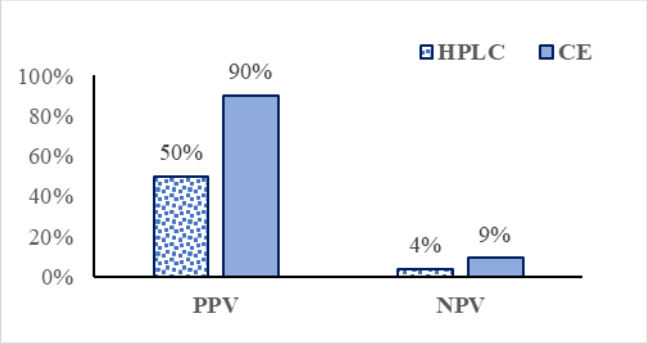

Figure 2: Comparison between Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of HPLC and CE.

Based on the findings shown in Figure 2, the PPV and NPV of HPLC were 50.0 % and 3.6%, respectively while PPV and NPV of CE were 90.0% and 9.1%, respectively. The PPV of HPLC reflected that there is 50.0% probability for a patient having HbCS to be detected as positive by HPLC. While based on the NPV, there is only 3.6% probability for patients without this abnormal haemoglobin to be detected as negative. Previous study by Wisedpanichkij et al. [8] found that the PPV and NPV of HPLC was 98.73% and 99.00%, respectively. Therefore, the results obtained in the present study were lower compared to the earlier study. As for CE, there is 90.0% probability for patients with HbCS to show positive result in the screening test while there is only 9.1% probability for patients without HbCS to be detected as negative when using CE. Therefore, PPV and NPV of CE showed higher percentages compared to that of HPLC. This outcome supports an earlier study conducted by Waneesorn et al. [5] which suggested that CE was more superior than HPLC in detecting HbCS.

4. CONCLUSION

Based on the current study, the sensitivity and specificity of CE appeared to be higher than the sensitivity and specificity of HPLC. Similarly, the PPV and NPV of CE demonstrated higher percentages than PPV and NPV of HPLC. Therefore, the findings of the present study reflected that CE was superior to HPLC. Hence, it is suggested to use CE as the screening method prior to molecular analysis in the detection of HbCS. As a recommendation for further study, the sample size should be increased to obtain sufficient amount of data especially for positive HbCS cases in order to achieve more valid results.

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