

A rare haemoglobin variant (Hb Phnom Penh) manifesting as a falsely high haemoglobin A1c value on ion-exchange chromatography

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ABSTRACT Most haemoglobin (Hb) variants are clinically silent. However, some Hb variants may interfere with the measurement of haemoglobin A1c (HbA1c), resulting in spurious values depending on the assays used. We herein report the case of a 53-year-old Taiwanese man with type 2 diabetes mellitus, who presented with an abnormal HbA1c peak on ion-exchange chromatography. Additional investigations, including intensified self-monitored blood glucose tests, an alternative HbA1c assay, and a glycaemic indicator based on a different method, revealed that the HbA1c values were falsely elevated. Subsequent DNA analysis confirmed that the patient was heterozygous for the insertion of an isoleucine residue at codons 117/118 of the α 1-globin gene, Hb Phnom Penh. Clinical laboratorians should be aware of the interfering factors in their HbA1c analysis. Cautious inspection of the chromatogram may provide a valuable clue to the presence of an Hb variant.

Keywords: diabetes mellitus, haemoglobin variant, Hb Phnom Penh, HbA1c

INTRODUCTION

Haemoglobin A1c (HbA1c) measurement is the gold standard for assessment of glycaemic control. In 2010, major international guidelines further recommended HbA1c as a measure for screening and diagnosing diabetes mellitus, and since then, the use of HbA1c test has substantially increased worldwide.^(1,2) However, there are several limitations to the measurement of HbA1c. Alterations in erythrocyte lifespan may cause a spurious HbA1c test result due to a change in Hb exposure in circulating glucose. For example, a falsely low HbA1c value could be a result of shortened erythrocyte survival due to haemolytic anaemia. Moreover, genetic variants of haemoglobin (Hb) may also affect the accuracy of HbA1c determination with different HbA1c assays.⁽³⁾ Previous case studies have reported subjects with extremely high (> 15%) or very low (below nondiabetic range) HbA1c values, which alerted physicians to the presence of an Hb variant.^(4,5) Nevertheless, even a small discrepancy between the HbA1c value and blood glucose levels can lead to considerable error in the management of diabetes mellitus. Furthermore, although common Hb variants may be automatically detected by some HbA1c analysers, identification of a rare Hb variant usually requires additional investigations.

We herein report Hb Phnom Penh, a rare Hb variant, in a Taiwanese man with type 2 diabetes mellitus. To our knowledge, this case is the first to demonstrate that this clinically silent Hb variant causes spuriously high HbA1c values, measured using ion-exchange high-performance liquid chromatography (HPLC).

CASE REPORT

A 53-year-old man with a medical history of dyslipidaemia, hypertension and type 2 diabetes mellitus presented to our

diabetes clinic for glucose control. The initial HbA1c test using ion-exchange HPLC showed a value of 8.7% and the simultaneously measured fasting blood glucose level was 133 mg/dL (or 7.4 mmol/L). In view of these values, we intensified the patient's diet and exercise education, adjusted his dosage of oral antidiabetic drugs, and suggested that self-monitoring of blood glucose be initiated. Three months later, the follow-up HbA1c value was 7.6%, self-monitored fasting blood glucose level was 95–100 mg/dL (or 5.3–5.6 mmol/L), and postprandial blood glucose level was 110–205 mg/dL (or 6.1–11.4 mmol/L). Based on these results, we initially concluded that the patient's glucose control was improving but not achieving the desired goal. However, the clinical laboratorian informed us of a probable inaccurate HbA1c value, as an aberrant A1c peak was found on the chromatogram (Fig. 1). To determine the interfering factor, complete blood cell count and Hb electrophoresis were performed. The peripheral blood indices showed the following: Hb level 15.0 g/dL with a red cell count of $4.7 \times 10^6/\mu\text{L}$; mean corpuscular volume 94.4 fL; and mean corpuscular Hb 31.9 pg. Nevertheless, Hb analysis by capillary electrophoresis revealed a normal pattern, quantifying HbA at 97.7% and HbA2 at 2.3%.

To further investigate and assess the accuracy of the HbA1c determination, we obtained the patient's blood samples at his subsequent visits. In one sample, the follow-up HbA1c test result from the ion-exchange HPLC analyser revealed an HbA1c level of 8.2%, while the boronate affinity chromatography result revealed an HbA1c level of 6.2%. In another sample, while the ion-exchange HPLC analyser result showed an HbA1c level of 7.5%, the fructosamine test result revealed a value of 239 $\mu\text{mol/L}$ (normal range < 285 $\mu\text{mol/L}$), which approximated to an HbA1c value of 5.7%. In the preceding three months, self-monitored

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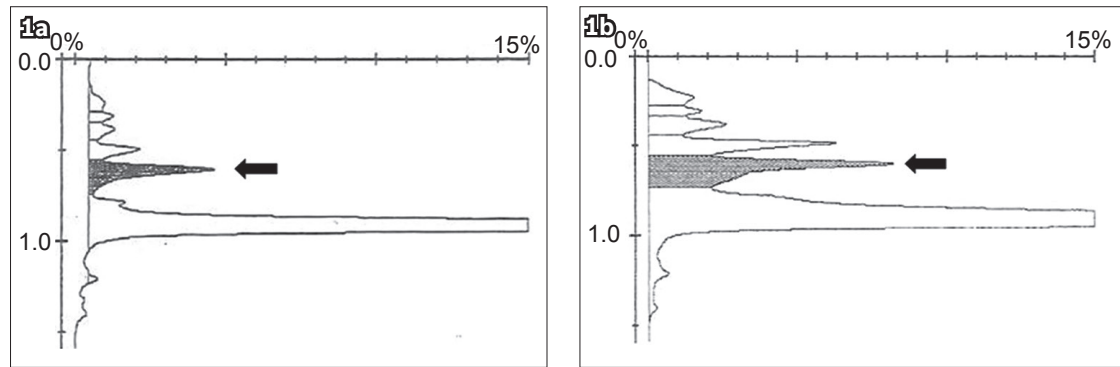


Fig. 1 (a) HLC-723 G8 chromatogram shows several peaks, including A1a, A1b, F, labile A1c, stable A1c (arrow) and A0; and (b) chromatogram of the patient with Hb Phnom Penh shows the aberrant A1c peak (arrow).

fasting blood glucose level was 90–100 mg/dL (or 5–5.6 mmol/L), and postprandial blood glucose level was 100–150 mg/dL (or 5.6–8.3 mmol/L). Taken together, both alternative tests showed more consistent results with measured blood glucose levels. These findings suggest that the HbA1c values measured on ion-exchange HPLC were inappropriately high.

To confirm the presence of an Hb variant, we eventually sent the patient's blood sample for genetic studies. Hb analysis performed using the HPLC method revealed an Hb variant (HbX 11.3%). Direct DNA sequence analysis confirmed that the patient was heterozygous for the insertion of an isoleucine residue between codons 117/118 of the α 1-globin gene, Hb Phnom Penh (HBA1:p.Phe118_Thr119insIle).

DISCUSSION

Hb Phnom Penh, a rare variant of Hb, was first described in a Cambodian child and his father.⁽⁶⁾ Thereafter, Hb Phnom Penh was reported in the Chinese, Taiwanese and Thais.^(7–9) By means of isopropanol precipitation and heat instability tests, Hb Phnom Penh has been proven to be a nonpathological variant. In previously reported cases, both patients showed the interaction of this α -chain variant with a common Southeast Asian deletion mutation.^(7,9) Routine blood tests revealed microcytic anaemia, alerting physicians to this genetic variant associated with thalassaemia. However, there is no report addressing the impact of this silent Hb variant on the measurement of HbA1c. Our patient with type 2 diabetes mellitus was not anaemic, and his genetic screening excluded other associated haemoglobin gene disorders. The present case is thus a unique phenotypic characterisation of Hb Phnom Penh in a patient with type 2 diabetes mellitus. Moreover, based on additional investigations, this case also demonstrated, for the first time, that Hb Phnom Penh caused a spuriously high HbA1c test result on ion-exchange HPLC.

Apart from blood glucose concentrations, the HbA1c value can be adversely affected by the presence of an Hb variant. The impact of genetic variants on HbA1c determination varies considerably among commercially available methods.⁽³⁾ Currently, there are more than 30 different glycohaemoglobin assay methods. The principle of HbA1c analysis can be divided into two groups – ion-exchange HPLC and electrophoresis are

assays based on molecular charge, while immunoassay and boronate affinity chromatography are methods using molecular structure. Among these assay methods, ion-exchange HPLC tends to demonstrate the greatest interference from the presence of Hb variants and derivatives on HbA1c values.⁽¹⁰⁾ In general, inaccurate HbA1c values occur when the Hb variant or its glycated derivative cannot be separated from HbA or HbA1c. In the present case, the aberrant peak was presumed to be the result of the Hb variant co-eluting with HbA1c. Boronate affinity chromatography, on the other hand, measures glycated Hb based on the interaction between cis-glycols and boronic acid. This method is well documented to be the HbA1c assay with the least interference for subjects with an Hb variant.^(3,10) However, as boronate affinity chromatography is unable to detect the presence of an Hb variant, it is not used as the first-line HbA1c analyser in our hospital. In the present case, the HbA1c value determined by boronate affinity chromatography was more consistent with blood glucose levels. This comparison not only reveals the nature of an inappropriately high HbA1c value measured using ion-exchange HPLC, but also provides a clue to the presence of an Hb variant.

Alternative assessment of glycaemic control using different methods such as the fructosamine test may be employed when the HbA1c test result is unreliable. Since the measurement of fructosamine depends on the glycation of serum proteins, it would not be influenced by Hb variants. In the present case, the fructosamine level was more consistent with the measured blood glucose concentrations than the HbA1c value measured using the HLC-723 G8 analyser. However, albumin with a shorter turnover of about two weeks leads to a need for frequent measurements. In addition, higher within-individual variation due to instability and inadequate evidence of its efficacy in predicting the risk for diabetic complications have excluded its use as a routine glycaemic index.⁽¹¹⁾

There are more than 1,100 Hb variants reported worldwide.⁽¹²⁾ Since HbA1c is the product of nonenzymatic glycation at one or both of the N-terminal valines of the Hb β -chain, most Hb variants affecting the measurement of HbA1c are due to mutations in the β -globin gene. However, some α -chain Hb variants have been reported to cause spurious HbA1c test results.^(13,14) In Taiwan, Hb J is identified as a common cause of falsely low HbA1c values

on the HLC-723 G8 analyser. In contrast, Hb E and Hb H have been demonstrated to cause spuriously high HbA1c values on the Bio-Rad Variant™ II Turbo analyser.^(1,5) This case report provides a new instance of a patient presenting with an inordinately high HbA1c value on HPLC-based assay.

Hypoglycaemia is associated with significant morbidity and mortality. Therefore, it is important for physicians to be aware that aggressive adjustment of antidiabetic medication based solely on falsely high HbA1c test results may lead to an increased risk of hypoglycaemia in patients. In the present case, frequent capillary glucose monitoring combined with boronate affinity chromatography seems to be the best alternative for glycaemic assessment.

In conclusion, we identified a rare and silent α -chain Hb variant, Hb Phnom Penh, which interfered with the measurement of HbA1c, leading to spuriously high HbA1c values. Clinicians and laboratorians should be aware of the limitations of HbA1c assays. In addition to unusually high or low HbA1c values, they should also carefully inspect chromatograms from ion-exchange HPLC to identify aberrant peaks, as this may help to detect the presence of an Hb variant. Further investigations, including an alternative HbA1c assay and glycaemic indicator based on different methods, should be used to clarify the nature of interference. DNA analysis is usually required to confirm a rare Hb variant.

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