

# HbG-Coushatta: An unexpected discovery during HbA1c measurement

Xinqi Cheng, Ming Li, Jie Wu, Wei Su

Department of Clinical Lab, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100730, China

## Background

HbA1c, the glycated fraction of hemoglobin A, is formed via non enzymatic glycation of the valine residue at the N-terminal of Hb  $\beta$  chain with glucose. It indicates the average blood glucose level over three months prior to the measurement. This measurement is widely used as the gold standard for blood glucose level monitoring in diabetic patients [1]. To date, various methods are being used in clinical laboratories for HbA1c measurement, including cation exchange-high performance liquid chromatography (HPLC), capillary electrophoresis, boronate affinity chromatography, and immunoassay. As the underlying principles are different among various methods used for HbA1c measurement, it is reasonable to suppose that the interference of Hb variants on HbA1c measurement could also be different.

## Methods

A 55-year old woman with type 2 diabetes and hyperlipidemia was prescribed with a long-term therapy of oral anti-diabetic medication, and monitoring of blood glucose and HbA1c was performed routinely. In the past two years, the patient's blood glucose values (6.8–7.8 mmol/l) were always higher than the upper limit of the reference interval (reference interval 3.6–6.1 mmol/l). However, her serials of results of blood HbA1c obtained from Variant II HbA1c HPLC system (Bio-Rad, USA) were quite stable (5.3%–5.5% 34.4–36.6 mmol/mol) [reference interval 4.5–6.3% (25.7–45.4 mmol/mol)]. The most recent laboratory tests showed that the patient had an elevated fasting blood glucose level (6.8 mmol/l), an elevated glycated albumin level (17.4%, reference interval 11.0–16.0%), but a decreased HbA1c level of 4.4% (24.6 mmol/mol) [reference interval 4.5–6.3% (25.7–45.4 mmol/mol)]. All the HbA1c results of this patient were in error. Review of the chromatograms in this case indicated clearly that there was interference in quantitation of hemoglobin A0 due to an unknown hemoglobin with an elution time close to that of hemoglobin A0 (Fig. 1).

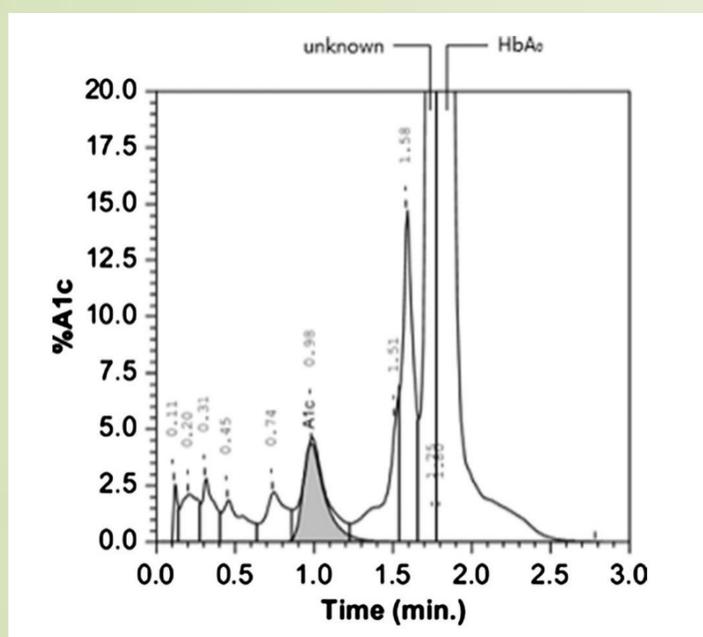


Fig. 1. HPLC chromatogram for HbA1c analysis. The retention time of unknown peak was 1.75 min. It was not completely separated from HbA0. The proportion of HbA1c analyzed by HPLC was 4.4% (24.6 mmol/mol).

The patient's HbA1c was evaluated by capillary electrophoresis (CAPILLARYS 2, Sebia, France) which has a strong power of discrimination for various Hb variants. To confirm the presence of an Hb variant in this patient, we profiled Hb through capillary electrophoresis (MINICAP, Sebia, France). Moreover, DNA sequencing of the Hb  $\beta$ -chain gene was performed to define its genotype. To evaluate the interference of the Hb variant on different methods of HbA1c measurements, we assessed the level of HbA1c in this patient by capillary electrophoresis and tandem HPLC-capillary electrophoresis (LC/CE) (Shanghai IFCC Reference Laboratory), one of the two IFCC reference methods, in which HbA1c and HbA0 are digested by protein endopeptidase at the site of amino acid 6 of the Hb  $\beta$  chain N terminus to form glycated hexapeptides (HbA1c) and non-glycated hexapeptides (HbA0), respectively. Then these two hexapeptides are separated and measured by LC/CE. HbA1c is defined as the ratio between the HbA1c concentration and the sum of HbA1c and HbA0 concentrations.

## Results

As expected, an Hb variant was confirmed by capillary electrophoresis, with the presence of an abnormal peak in the HbD zone, with a proportion of 43.3%. Further DNA sequencing of the Hb  $\beta$ -chain gene (HBB) revealed an alteration at codon 22 (GAA to GCA), resulting in an amino acid change from glutamic acid to alanine, which was previously named as HbG Coushatta. The measurement of HbA1c through capillary electrophoresis yielded a result of 7.7% (60.6 mmol/mol) (Fig.2). Tandem HPLC-capillary electrophoresis (LC/CE) (Shanghai IFCC reference laboratory), reported an HbA1c value of 6.7% (49.7 mmol/mol).

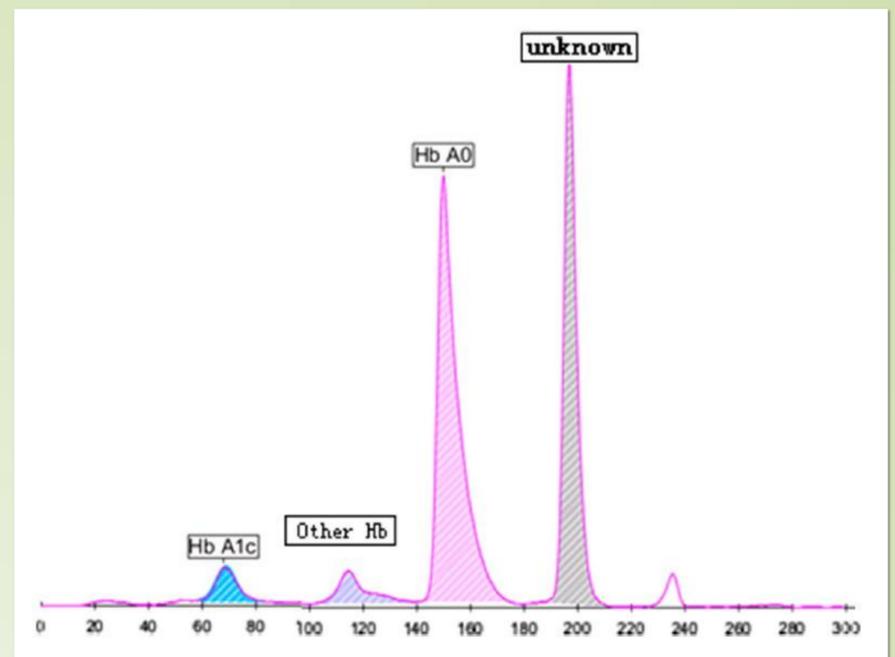


Fig. 2. HbA1c assay by capillary electrophoresis. An unknown peak appears between HbA0 and HbA2, which corresponds to the non-glycated form of the variant (HbG0). HbG0 was completely separated from HbA0; while the glycated form of the variant (HbG1c) is also separated as it appears between HbA1c and HbA0. The proportion of HbA1c analyzed by capillary electrophoresis was 7.7% (60.6 mmol/mol).

## Discussion

Our results demonstrated that an Hb variant, namely HbG Coushatta, interfered with the initial assessment of HbA1c with the HPLC method. The HbG Coushatta, which is encountered in this case, is a variant that is frequently found in China [2]. In the HbA1c analysis using the HPLC, an additional peak from HbG Coushatta variant was observed, but it was not completely separated from HbA0. It was difficult to identify the exact position of HbA0 peak and assess the separation between HbA0 and HbG Coushatta on the Variant II chromatogram, thus we suspected that the peak area under the HbA0 could not be integrated accurately, resulting in falsely low HbA1c result. An unsatisfactory separation of the Hb variants and HbA0 or HbA1c is the main reason for the inaccurate HbA1c measurement with HPLC. The resolution of capillary electrophoresis is superior to HPLC, allowing the separation of many common and rare Hb variants from the HbA0 fraction [3]. In the present case, HbG Coushatta was completely separated from HbA1c and HbA0 fractions with capillary electrophoresis, which could not be achieved by HPLC. However, the HbA1c result provided by capillary electrophoresis analysis, 7.7% (60.6 mmol/mol), was quite different from that of IFCC reference method, 6.7% (49.7 mmol/mol). This may be due to the position of variant-specific mutation in HbG Coushatta (amino acid 22 from N-terminal of Hb  $\beta$  chain). When digested at amino acid 6 with the IFCC reference method, the mutated  $\beta$ -globin chain produces the same hexapeptides as HbA0. Therefore, hexapeptides derived from HbG Coushatta are likely to be involved in the calculation of HbA1c, which indicates that the IFCC reference method could not distinguish HbG0 from HbA0 and HbG1c from HbA1c. The glycation rate of a variant is not always the same as that of HbA. For example, other variants, such as Hb Himeji, can have higher glycation rate than HbA. This increased the proportion of HbA1c although the patient's serum glucose is normal [4]. In this case, results analysis of integrals from the capillary electrophoregram suggested that the glycation rate of HbG Coushatta was less than that of HbA. A difference in glycation rates could lead to an underestimate of HbA1c as determined by the IFCC reference method, because that method reflects the sum of both HbA1c and HbG1c.

## Conclusions

Although HPLC is widely used in routine laboratories, many substances could produce interference on this method. Commonly encountered ones in clinical practice include Hb variants, carbamylated hemoglobin (found in patients with renal failure) and HbF (increased in  $\beta$ -thalassemia, hereditary persistence of foetal hemoglobin HPFH, or in neonates). Therefore measurement of HbA1c by HPLC may not be suitable for such conditions. Alternately, other measurement methods, as the capillary electrophoresis used in this report, should be applied. This case tells us that elimination of Hb variant interference is critical to achieve an accurate result of HbA1c. High resolution methods as capillary electrophoresis might be preferred in the regions with high prevalence of Hb variants.

[1] Jeppsson JO, Kobold U, Barr J, et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002;40:78–89.  
[2] Peng J, Gong WX, Huang CZ. Research progress of hemoglobin G disease. Med Recapitul 2011;17:1948–50.



[3] Urrechaga E. High resolution HbA1c separation and hemoglobinopathy detection by capillary electrophoresis. Am J Clin Pathol 2012;138:448–56.  
[4] Ohba Y, Miyaji T, Murakami M, et al. Hb Himeji or beta 140 (H18) Ala-Asp. A slightly unstable hemoglobin with increased beta N-terminal glycation. Hemoglobin 1986; 10:109–26.