

Visual inspection of chromatograms assist interpretation of glycated hemoglobin (HbA1c): a case report

Running title: Chromatograms to assist HbA1c interpretation

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CASE SUMMARY

- 58-year-old Chinese female with clinically silent hemoglobinopathy
- Normal glucose tolerance: fasting plasma glucose 5.6mmol/L, plasma glucose two hours after 75g glucose loading 5.98mmol/L
- Abnormally low, implausible glycated hemoglobin (HbA1c) results: 2.0%, 3.6% (16mmol/mol) on retesting
- Normal hemoglobin level with increased turnover: hemoglobin 136g/L, reticulocyte percentage 6.53%
- High-performance liquid chromatography (HPLC) indicated an abnormal peak
- Heterozygous mutation in the HBB gene: c.242T>A, Leu81His
- Same mutation and consistent phenotype in the daughter of the proband
- HbA1c values estimated by HPLC is unreliable in patients with hemoglobinopathies, but visual inspection of the chromatogram can be used to identify hemoglobinopathies

CASE NARRATIVE

A 58-year-old woman presented with elevated fasting plasma glucose (FPG) found in a medical check-up. She was asymptomatic and clinical examination was normal apart from slight sclera jaundice. The 75-g oral glucose tolerance test (OGTT) showed FPG of 5.6mmol/L and 2-hour postprandial plasma glucose of 5.98mmol/L. The glycated hemoglobin (HbA1c) taken at the same visit was 2.0% (value unavailable in the International Federation of Clinical Chemistry [IFCC] unit, assayed by the Tosoh G8 analyzer), and was 3.6% (16mmol/mol) on retesting (assayed by the Bio-Rad D-10 TM analyzer). The synchronous glycated albumin (GA) was 11.59%. Her other blood results were: hemoglobin (Hb) 136g/L, red blood cell (RBC) count $4.35 \times 10^{12}/L$, mean corpuscular volume 98.9fL (normal range, 82-100), mean corpuscular hemoglobin 31.3pg (normal range 27-34), and

mean corpuscular haemoglobin concentration 316g/L (normal range 316-354). Further investigations showed elevated reticulocytes, elevated serum indirect bilirubin (indirect bilirubin: 26.9 μ mol/L, normal range <20; direct bilirubin: 12.6 μ mol/L, normal range <8.8; total bilirubin: 39.5 μ mol/L, normal range 5-28), normal transaminases, and negative fecal occult blood test. Haptoglobin was significantly decreased (<58.3mg/L) and methemoglobin reduction test was 7.7%, indicating hemolysis. The direct Coombs test was negative, and glucose-6-phosphate dehydrogenase activity was normal. Hb electrophoresis revealed two abnormal bands (assayed by Sebia CAPILLARYS 2), so hemoglobinopathy was suspected. Gene sequencing identified a heterozygous mutation (c.242T>A, Leu81His) in the HBB gene (1). During the family screening, the daughter of the proband was found to have normal blood glucose, decreased HbA1c (3.9%, 19mmol/mol) with abnormal bands on Hb electrophoresis tracing, and the same mutation (pedigree presented as Supplemental Figure S1). GA was used to monitor the patient's glucose level afterward.

When reviewing the case, we further requested the high-performance liquid chromatography (HPLC) chromatogram of this patient, in which an abnormal peak was presented (arrowed, Figure 1A) and was reported as the variant window. This indicated the capability of HPLC chromatograms in identifying unexpected variants. The HPLC chromatogram of the patient's husband was presented as normal control (Figure 1B, both assayed by the Bio-Rad D-10 TM analyser).

HbA1c is widely used as an independent diagnostic criterion for diabetes and as a monitor for glycemic control (2-4). However, under several circumstances, HbA1c by itself is not able to accurately reflect the plasma glucose level (5, 6). International guidelines have been calling clinicians' attention to several comorbid conditions when HbA1c is unreliable, such as

hemoglobinopathy and hemolytic anemia (2). In the presented case, an increased erythrocyte turnover and shortened RBC lifespan resulted in the abnormally decreased HbA1c reading. The majority of assay methods certified by the National Glycohemoglobin Standardization Program (NGSP) are not affected by common Hb traits (7), but if an Hb variant is detected, additional workup to further characterize the Hb variant may be necessary, and results should be correlated with complete blood count data and with clinical findings.

HPLC is one of the common assays for HbA1c measurement (8), and abnormal HPLC chromatograms may inform clinicians and laboratory staff of potential interferences. In the presented case, the HPLC chromatogram was requested post hoc because of the extremely abnormal HbA1c value, but could be easily overlooked if the hemoglobin variant caused only mild interference. Although the necessity of presenting chromatograms routinely to clinicians needs further discussion, it is acknowledged that when measuring HbA1c by a chromatographic technique, visual inspection of chromatograms prior to reporting of HbA1c results is warranted, especially when there is discordance between HbA1c and other clinical data.

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Author contributions

Qianrui Li and Sheyu Li composed the manuscript. Yuling Xiao interpreted the laboratory results. Anoop Dinesh Shah critically reviewed the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

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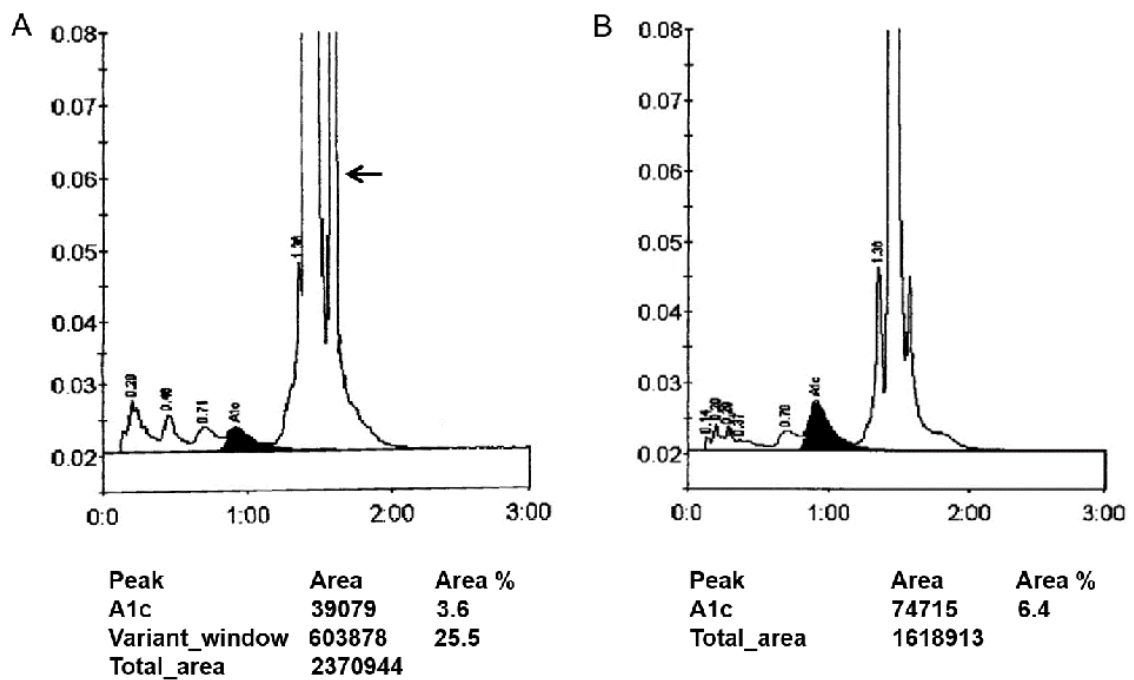
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Figure 1. Chromatograms of glycated hemoglobin (HbA1c) tests.

1A. High-performance liquid chromatography (HPLC) chromatogram of the proband. Arrow indicated the abnormal peak. The glycated hemoglobin (HbA1c) measured was 3.6% (16mmol/mol).

1B. HPLC chromatogram of husband of the proband as normal control. The glycated hemoglobin (HbA1c) measured was 6.4% (47mmol/mol).



Supplementary Figure S1. Pedigree of the family.

Arrow indicated the proband.

