Visual Inspection of Chromatograms Assists Interpretation of HbA_{1c}: A Case Report

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CASE SUMMARY

- 58-year-old Chinese female with clinically silent hemoglobinopathy
- Normal glucose tolerance: fasting plasma glucose 5.6 mmol/L, plasma glucose 2 h after 75-g glucose loading 5.98 mmol/L
- Abnormally low, implausible HbA_{1c} results: 2.0%; 3.6% (16 mmol/mol) on retesting
- Normal hemoglobin (Hb) level with increased turnover: Hb 136 g/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
- HbA_{1c} values estimated by HPLC are 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 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 showed fasting plasma glucose of 5.6 mmol/L and 2-h postprandial plasma glucose of 5.98 mmol/L. The HbA_{1c} taken at the same visit was 2.0% (value unavailable in International Federation of Clinical Chemistry and Laboratory Medicine [IFCC] units) (assayed by the Tosoh G8 analyzer); on retesting, it was 3.6% (16 mmol/mol) (assayed by the Bio-Rad D-10 TM analyzer). The synchronous glycated albumin was 11.59%. Her other blood results were as follows: Hb 136 g/L, red blood cell count 4.35×10^{12}/L, mean corpuscular volume 98.9 fL (normal range 82–100), mean corpuscular Hb 31.3 pg (normal range 27–34), and mean corpuscular Hb concentration 316 g/L (normal range 316–354). Further investigations showed elevated reticulocytes, elevated serum indirect bilirubin (indirect bilirubin 26.9 μmol/L, normal range <20; direct bilirubin 12.6 μmol/L, normal range <8.8; total bilirubin 39.5 μmol/L, normal range 5–28), normal transaminases, and negative fecal occult blood test. Haptoglobin was significantly decreased (<58.3 mg/L) and the 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 family screening, the daughter of the proband was found to have normal blood glucose, decreased HbA_{1c} (3.9%, 19 mmol/mol) with abnormal bands on Hb electrophoresis tracing, and the same mutation (pedigree presented as Supplementary Fig. 1). Glycated albumin was used to monitor the patient's glucose level afterward.

When reviewing the case, we further requested the HPLC chromatogram of this patient, in which an abnormal peak was presented (arrow, Fig. 1*A*) 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 served as a normal control (Fig. 1*B*; both assayed by the Bio-Rad D-10 TM analyzer).

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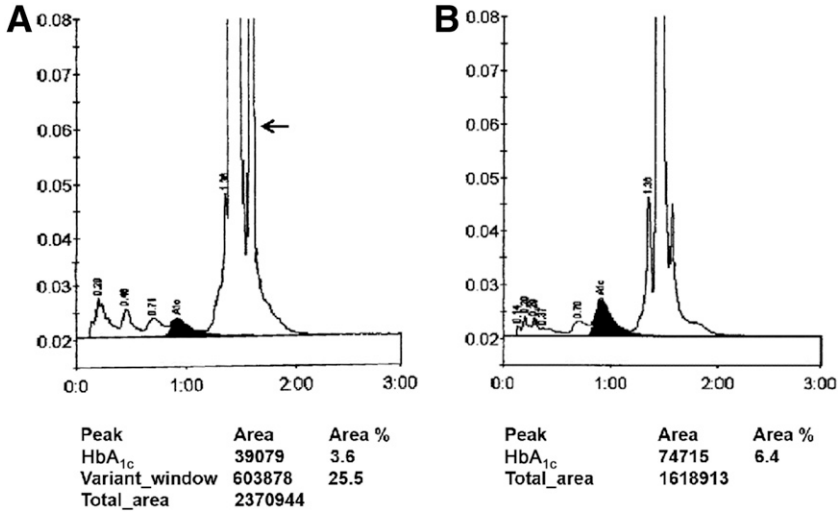

Figure 1—Chromatograms of HbA_{1c} tests. *A*: HPLC chromatogram of the proband. Arrow indicates the abnormal peak. The HbA_{1c} measured was 3.6% (16 mmol/mol). *B*: HPLC chromatogram of the husband of the proband as normal control. The HbA_{1c} measured was 6.4% (47 mmol/mol).

HbA_{1c} is widely used as an independent diagnostic criterion for diabetes and as a monitor for glycemic control (2–4). However, under several circumstances, HbA_{1c} by itself does not accurately reflect the plasma glucose level (5,6). International guidelines have called clinicians' attention to several comorbid conditions in which HbA_{1c} is unreliable, such as hemoglobinopathy and hemolytic anemia (2). In the presented case, an increased erythrocyte turnover and shortened red blood cell life span resulted in the abnormally decreased HbA_{1c} reading. The majority of assay methods certified by the NGSP (formerly, National Glycohemoglobin Standardization Program) 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 HbA_{1c} 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 HbA_{1c} value, but it could easily be 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 HbA_{1c} by a chromatographic technique, visual inspection of chromatograms prior to reporting of HbA_{1c} results is warranted, especially when there is discordance between HbA_{1c} and other clinical data.

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