Unexpected Hemoglobin A₁c Results

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²Nonstandard abbreviations: Hb, hemoglobin; CE-HPLC, cation-exchange high performance liquid chromatography; Hb S, sickle cell Hb.

CASE

A 52-year-old woman with a medical history of hepatitis B, hyperlipidemia, hypertension, anemia, and depression presented to the internal medicine clinic for a routine visit. Laboratory tests 3 months previously had revealed an impaired fasting glucose concentration of 5.9 mmol/L (106 mg/dL) [reference interval, 3.9 –5.6 mmol/L (70 –100 mg/dL)]. Therefore, a hemoglobin (Hb)² A₁c analysis was performed. The initial Hb A₁c evaluation by cation-exchange HPLC (CE-HPLC) (Hb A₁c Program on the VARIANT II TURBOlink System; Bio-Rad Laboratories) showed an Hb A₁c value of 115.8% (reference interval, 4.0%–6.0%) (Fig. 1). In an effort to determine if the unusual Hb A₁c result was due to potential hemoglobinopathies, we performed an Hb variant analysis with the Bio-Rad VARIANT CE-HPLC β-Thanassemia Short Program. The analysis revealed the absence of Hb A and the presence of sickle cell Hb (Hb S) (37.4%), along with normal Hb A₂ (3.2%) and Hb F (<1.0%) (Fig. 2). Also evident was another large peak (53.0%) that eluted earlier than Hb A, which we called P₂. This study suggested the presence of an Hb variant with a chromatographic retention time virtually identical to that of Hb A₁c, in addition to Hb S (Figs. 1 and 2). A subsequent Hb electrophoretic analysis at pH 6.0 (QuickGel Acid; Helena Laboratories) identified Hb S and another abnormal band with a mobility similar to Hb F (not shown).

PATIENT FOLLOW-UP

To identify the Hb variants, we investigated DNA sequences corresponding to the patient’s β-globin genes. This analysis identified a substitution at codon 6 [GAG to GTG (Glu to Val)] on one allele, corresponding to Hb S, and a substitution at codon 1 [GTG to GCG (Val to Ala)] on the other allele, corresponding to Hb Raleigh. The presence of these hemoglobinopathies suggested that the spurious HbA₁c result obtained with the CE-HPLC method was due to the elution of Hb Raleigh, which has a retention time similar to that of Hb A₁c. We evaluated the Hb A₁c result with a turbidimetric inhibition immunoassay (Dimension® Clinical Chemistry System; Siemens) and obtained an Hb A₁c value of 4.1%, which was not consistent with the impaired fasting glucose concentration of 5.9 mmol/L (106 mg/dL).
Clinical Case Study

![Fig. 1. CE-HPLC chromatogram for Hb A1c analysis. Hb S and an aberrant Hb A1c value of 115.8% represented the predominant Hb peaks in the chromatogram.](image1)

<table>
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<th>Percent</th>
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<th>Area</th>
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<td>33627</td>
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<tr>
<td>F</td>
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*Value outside of expected range: Total area = 1656306

![Fig. 2. Chromatogram of Hb variants analysis with the CE-HPLC β-Thalassemia Short Program. Hb S (37.4%), wild-type Hb A2 (3.2%), and Hb F (<1.0%) were identified, but Hb A was not detected. A large peak, which we designated P2, was detected at 53.0%.](image2)

Questions to Consider

- What are the various types of methods used for measuring Hb A1c?
- How do Hb variants interfere with each of these Hb A1c methods?
- What actions should be taken when a spurious Hb A1c result is present?

Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the February 2011 issue of *Clinical Chemistry*. To view the case and comments online, go to [http://wwwclinchem.org/content/vol57/issue2](http://wwwclinchem.org/content/vol57/issue2) and follow the link to the Clinical Case Study and Commentaries.

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