Incidental Findings of Variant Hemoglobin During Hemoglobin A1c Testing

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Abstract

A variant hemoglobin fraction may be an incidental finding during HbA1c analysis using the G8 Tosoh HPLC analyzer, but it is unclear if the retention times and fraction patterns can reliably predict the findings of a high-performance liquid chromatography (HPLC) β-thalassemia program (Bio Rad Variant II analyzer). We chose 100 samples sent for HbA1c determinations (G8 Tosoh) with an incidental finding of variant hemoglobin and did a reflex test using the Bio Rad Variant II analyzer (β-thalassemia program). Two observers attempted to predict the results with that analyzer from fraction patterns and retention times of the hemoglobin variants detected with the G8. They independently identified all hemoglobin variants (HbS, Hb Setif, HbC, and HbD) by their patterns and retention times. We conclude that HPLC confirmation of certain variant hemoglobin fractions found incidentally during HbA1c testing on the G8 Tosoh is not necessary.

Measurement of glycated proteins, primarily hemoglobin (Hb) A1c, is widely used for routine monitoring of glucose in patients with diabetes and was recently approved for diabetes screening. HbA1c measured with the high-performance liquid chromatography (HPLC) G8 system (HbA1c variant mode, Tosoh Corporation, Tokyo, Japan) is directly traceable to the International Federation of Clinical Chemistry reference standard method and has excellent accuracy and precision.1 Hemoglobin variants such as HbS, HbC, and HbD have different retention times than HbA and do not interfere with the HbA1c measurements, but it is unclear if the results are concordant with HPLC results using a β-thalassemia program (Variant II analyzer, Bio Rad Laboratories, Munich, Germany). If retention times and/or patterns of the variants are distinctive, the reporting of these incidental findings will provide a cost-effective way to identify carriers without the need for reflex HPLC testing to screen for those variants. One study found that retention times with the G7 Analyzer (Tosoh) reliably differentiated among various hemoglobin variants2 but did not report the fraction pattern of the variant hemoglobin fractions.

We routinely identify hemoglobin variants in our region with an HPLC analyzer (Bio Rad Variant II) that is approved by the Food and Drug Administration (FDA) and widely used to screen for β-thalassemia minor. In this study, we attempt to predict the findings of the Variant II directly from the pattern and retention time of the fraction found incidentally during HbA1c determinations.

Materials and Methods

Our laboratory routinely tests about 800 whole blood samples per day for HbA1c using a G8 HPLC analyzer.
During a 2-month period we added an HPLC test using the Bio Rad Variant II analyzer to any sample that had hemoglobin fractions with abnormal retention times on the G8 Tosoh HbA1c chromatogram. The results for fractions found previously with the Bio Rad Variant II analyzer for HbS (~300), Hb Setif (n = 10), and HbD (few cases) were completely concordant with results of DNA sequencing. Some samples, however, were defined as HbC with the Variant analyzer but DNA sequencing identified HbO-Arab. No DNA sequencing was done in our study, and the results of the Bio Rad Variant II analyzer were used as the gold standard. We tested consecutive samples except for 3 archived historical samples with HbD. We excluded from this study samples with fractions that interfere with the HbA1c test, including primarily HbF and Hb Rambam, which has a distinctive pattern. Patients do not pay for their laboratory tests and reflex testing is at the discretion of the laboratory. The data for this study are part of our quality management system, and reflex testing is thought to represent good laboratory practice. The use of the data is in accordance with our institutions’ ethical standards.

Two of the authors (P.F. and C.H.) were blinded to the results of the Bio Rad Variant II, and attempted to differentiate the variant hemoglobin fractions according to their pattern and retention time. Means and ranges of the retention times were determined.

**Results**

Two of the authors (P.F. and C.H) independently identified all hemoglobin variants [Figure II]. HbS has a wide pattern with a small peak to the left of the large fraction. Hb Setif has a very narrow distribution pattern. HbC also has a
wide pattern but longer retention times, and HbD is wide and attached to the HbA fraction. One rare variant (Camden) was easily differentiated from the others but the observers were not familiar with its pattern. There was no overlap of the ranges for the various hemoglobin variant fractions [Table I].

Half of the Hb Setif variants were found in the same town; the prevalence was 7 of 493 tests (1.4%). In the various municipalities, the prevalence of HbS was 1.0% (3/291), 2.0% (3/151), 0.9% (5/538), 1.3% (9/714), 0.6% (2/342), 0.5% (2/403), 5.3% (6/114), 0.7% (10/1,385) and 0.6% (4/693) of the HbA1c tests.

Discussion

The main finding of our study was that we can reliably predict findings of the HPLC β-thalassemia program (Bio-Rad Variant II) based on the fraction patterns and retention times found incidentally on HbA1c determinations (G8 Tosoh). This study confirms the results of a previous study using the G7 analyzer, which reported unique retention times,

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>Number, %</th>
<th>Mean (Range) Retention Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>55</td>
<td>1.19 (1.17-1.20)</td>
</tr>
<tr>
<td>Setif</td>
<td>32</td>
<td>1.23 (1.21-1.24)</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>1.31 (1.31-1.33)</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>1.04 (1.01-1.06)</td>
</tr>
<tr>
<td>Camden</td>
<td>1</td>
<td>0.91 (0.91)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
but did not use the patterns of the fractions for identification. The retention times with the G7 analyzer for the various hemoglobin variants were different from those found with the G8 analyzer, but the order of the retention times was the same: the shortest time for HbD, then HbS, then Hb Setif, and finally HbC. In the study conducted using the G7 analyzer, the authors reported that 3 of 255 HbS carriers had outlying results believed to have occurred because of a calibration error; we did not observe any discordance.

An unexpected finding in our study was that Hb Setif made up a considerable proportion of our variant hemoglobins; in 1 town it was found in 1.7% of tests sent for HbA_{1c} analysis. Hb Setif results from an asparatic acid to tyrosine substitution at codon 94(GAC>TAC) of the α-globulin gene and is linked with in vitro pseudosickling. Twelve carriers were found in Western Iran and the prevalence of Hb Setif is 0.1% in 65,668 tests conducted in Cyprus, compared with 0.2% for HbS in the same population. However, the clinical significance and advisability of reporting the presence of Hb Setif is uncertain; a single report of Hb Setif combined with a highly unstable α-globulin variant found only mild microcytic anemia.

Laboratory practice in reporting incidental variants detected during HbA_{1c} testing varies: 1 study found that some laboratories mentioned the presence of a variant only if it interfered with the test, whereas others suggested follow-up including either sickle screens or hemoglobin electrophoresis. We believe that variant hemoglobins should be reported because they might affect clinical decisions. Our results, however, suggest that reflex HPLC testing using an HPLC β-thalassemia program is unnecessary because the presumptive identification of hemoglobin variants with an additional test is redundant and does not preclude the need for DNA confirmation.

A major limitation of this study is that we did not have DNA confirmation. However, the Bio-Rad analyzer is an appropriate reference for this study because it is FDA-approved for identifying HbA_{2} and HbF, provides “presumptive” identification of hemoglobin variants, and in our geographic area is completely concordant with DNA sequencing results for HbS, HbD, and Hb Setif. Nevertheless, we recommend reporting positive findings with either the G8 or Bio-Rad Variant II analyzers as presumptive and to conduct DNA sequencing for prenatal testing if both parents are suspected to be carriers of variants of hemoglobin found on any HPLC test, because results might not be 100% accurate. Extrapolation of our findings to other geographic areas should be done with caution.

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References