Effects of 7 Hemoglobin Variants on the Measurement of Glycohemoglobin by 14 Analytical Methods, Seung-Tae Lee,1 Cas W. Weykamp,2 Yong-Wha Lee,3 Jong-Won Kim,1 and Chang-Seok Ki1* (1 Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; 2 Department of Clinical Chemistry, Queen Beatrix Hospital, Winterswijk, The Netherlands; 3 Department of Laboratory Medicine and Genetics, Soonchunhyang University Bucheon Hospital and Soonchunhyang University College of Medicine, Gyeonggi-do, Korea; * address correspondence to this author at: Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-Dong, Gangnam-Gu, Seoul 135-710 Korea; fax 82-2-3410-2719, e-mail changski@skku.edu)

Background: Hemoglobin variants (HbVAR) are not uncommon in the Korean population, with Hb G-Coushatta and Hb Queens being the 2 most common HbVAR. Hb G-Coushatta is also the most common HbVAR in Chinese people from the Silk Road region, as well as in some North American Indian tribes. However, data are scarce on the effect of these HbVAR on the different methods for analyzing HbA1c.

Methods: Specimens from 24 individuals with 7 HbVAR (Hb G-Coushatta, Hb Queens, Hb G-Hsi-Tsou, Hb Ube-4, Hb G-Waimanalo, Hb Inglewood, and Hb Bologna-St. Orsola) were collected and tested using the International Federation of Clinical Chemistry primary reference method as well as 14 routine HbA1c assay methods.

Results: Hb G-Coushatta showed a clinically significant effect on the measured HbA1c, particularly when analysis was performed with ion-exchange HPLC methods with short elution times. This interference could be resolved by measuring the HbA1c using other methods such as HPLC with a long elution time, immunoassay, boronate affinity chromatography, and enzymatic assay. Hb Queens showed a clinically significant difference, defined as a >10% deviation from regression lines, in results from the 2 HPLC methods but not in the other methods. The remaining 5 rare HbVAR showed different HbA1c results in the different assays.

Conclusion: Hb G-Coushatta, Hb Queens, and other rare HbVAR can interfere with glycohemoglobin assays, including ion-exchange HPLC methods with short elution times, but the interference can be resolved using other unaffected methods. It is important to identify these HbVAR through a careful inspection of the chromatograms and apply other noninterfering methods for accurate measurements of the HbA1c.

The presence of variant hemoglobins (HbVAR) can interfere with some assays used for measuring glycohemoglobin (GHb) (1). Most of these HbVAR (as many as 800 types thus far) have been recognized in single reports of studies using 1 or 2 HbA1c determination methods. Nevertheless, studies examining the effects of these variants on various methods have been restricted to a few HbVAR such as HbS and HbC, which are common in some regional populations (2–5).

We previously reported that the HbVAR and high Hbf in the Korean population are not uncommon, with Hb G-Coushatta and Hb Queens being the 2 main HbVAR found in Koreans (6). Hb G-Coushatta is also one of the most common HbVAR found in families from the Silk Road region of China and some North American Indian tribes, as well as in Koreans, Japanese, Thais, Turks, Algerians, and Egyptians (6–10). Hb Queens is the most common α-chain variant in people from the Silk Road region of China, and has also been found in Koreans, Japanese, Vietnamese, and Thai families (6, 7, 10). However, there are limited data on the effect of these HbVAR on the different methods for analyzing the HbA1c. Therefore, we examined the possible effects on GHb results for Hb G-Coushatta and Hb Queens and 5 rare HbVAR analyzed with 14 different methods commonly used to analyze HbA1c. The Institutional Review Board of Samsung Medical Center, Seoul, Korea, approved this study.

Fresh whole blood specimens were obtained from 24 patients with 7 different HbVAR; 7 with Hb G-Coushatta [β 22(B4) Glu>Ala], 11 with Hb Queens [α 34(B15) Leu>Arg], 2 with Hb G-Hsi-Tsou [β 79(EF3) Asp>Gly], 1 each with Hb Ube-4 [α 116(GH4) Glu>Ala], Hb G-Waimanalo [α 64(E13) Asp>Asn], Hb Inglewood [β 142(H20) Ala>Thr], and Hb Bologna-St. Orsola [β 146(HC3) His>Tyr]. These variants were identified by direct sequencing of the hemoglobin, alpha 1 (HBA1); hemoglobin, alpha 2 (HBA2); and hemoglobin, beta (HBB) genes, using previously reported protocols (6). The samples were stored below −70 °C when the assay methods were unavailable within 7 days of collection.

We analyzed all the collected specimens using the International Federation of Clinical Chemistry (IFCC) primary reference method (PRM) with HPLC-capillary electrophoresis according to approved IFCC protocols (11). This method was chosen as a comparative method owing to its excellent precision and accuracy and minimal interference with common HbVAR, such as Hbs and HbC (11, 12). Furthermore, the values of IFCC PRM accorded very well with those of the boronate affinity method, which has been regarded as unaffected by most HbVAR (1). To compare the values with other methods, the results of the IFCC PRM were converted into a National GHb Standardization Program (NGSP) value using the following master equation suggested by Hoelzel et al. (12):

Abbreviations: Ultra2, Primus Ultra2; PDQ, Primus PDQ; VII, Bio-Rad Variant II; VII-T, Bio-Rad Variant II Turbo; VII-TM, Bio-Rad Variant II in thalassemia mode; G7-SM, Tosoh G7 in standard mode; G7-TM, Tosoh G7 in variant mode; HBA1c, Diabetes mellitus; Unimate, Roche Unimate HbA1c kit; TinaQuant-II, Roche TinaQuant II reagent; AU640, Olympus AU640; DCA2000, Siemens DCA 2000; Norudia, Daichi Norudia HbA1c, reagent.
NGSP – HbA1c = 0.915 (IFCC – HbA1c) + 2.15%.

We also analyzed the specimens with IFCC secondary reference methods (SRM), including Primus Ultra2 A1c and Hemoglobin Variants Analyzer (Ultra2), Roche Uni- mate HbA1c test reagents on the Modular system (Unimate), Tosoh G7 in variant mode (G7-VM), and an Arkray HA-8160 HbA1c analyzer in diabetic (HA8160-DM) and thalassemia mode (HA8160-TM). Other commercially available assays were also used to examine the GHb concentrations, including Primus PDQ (PDQ), Bio-Rad Variant II System (VII), Bio-Rad Variant II Turbo System (VII-T), Bio-Rad Variant II System in thalassemia mode (VII-TM), Tosoh G7 in standard mode (G7-SM), Roche TinaQuant II reagent on the Modular system (TinaQuant-II), Olympus AU640 System (AU640), Siemens DCA 2000 Analyzer (DCA2000), and Daiichi Norudia HbA1c test reagent on the Roche Modular system (Norudia). Inter-method calibration differences were corrected as previously reported (5).

We used EP Evaluator Release 7 software (RHOADS) to perform Deming regression analysis on the 2 common HbV AR (Hb G-Coushatta and Hb Queens) (13). The deviations at 6% and 7% HbA1c concentrations, at which the examined concentrations of the study samples were distributed, were predicted using the regression lines, and a deviation >10% of the assessing points (±0.6% and ±0.7%, respectively) was defined as a clinically significant difference (14).

The chromatogram results obtained with the 7 different HPLC methods for the 2 common HbV AR are shown in Fig. 1. Hb G-Coushatta was unrecognizable in VII and HA8160-DM, possibly owing to coelution with the normal peaks, whereas the glycated form of this HbV AR was eluted separately as a small peak between the S-A1c and A0 peaks in VII-T and G7-SM. Although no abnormal peaks occurred on the VII instrument, we noted blunting of the normal P3 peak and a subtle shift in the retention time of the A0 peak (from approximately 1.78 min to approximately 1.84 min). On the VII-T instrument, the variant peak might be mistaken for a normal P3 peak, which is sometimes observed, but the variant peak had a slightly shorter retention time (approximately 0.72 min) than the P3 peak (approximately 0.77 min; see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol53/issue12). Instruments with long elution times, including VII-TM, G7-VM, and HA8160-TM separated Hb G-Coushatta from the other normal peaks.

From the Deming regression analysis, Hb G-Coushatta was predicted to produce clinically significant negative biases in most ion-exchange HPLC methods at both 6% and 7% HbA1c, with the exception of VII-TM, which showed acceptable results (Table 1 and see Fig. 1 in the online Data Supplement). This underestimation of the HbA1c concentrations appears to be due to the coelution of the nonglycated HbV AR with the normal A0 peak, and the separate elution of the glycated HbV AR from the normal A1c peak. G7-VM, which separated both the glycated and nonglycated Hb G-Coushatta, did not automatically subtract the variant portion when calculating the HbA1c. When a manual calculation was performed by subtracting the proportion of the variant peak areas in the

![Fig. 1. Chromatograms of the 2 common HbV AR in the 7 different HPLC methods.](image)

The solid arrowheads indicate the HbV AR, and the open arrowheads indicate the glycated HbV AR. Hb G-Coushatta was unrecognizable in VII and HA8160-DM, and was recognized as only a small glycated peak between the S-A1c and A0 in VII-T and G7-SM, whereas the nonglycated form was separated from the other normal peaks in VII-TM, G7-VM, and HA8160-TM. The nonglycated form of Hb Queens was eluted discretely in all instruments except G7-SM.
denominator according to the manufacturer’s instructions, the differences fell into an acceptable mean difference (0.3%), but there were wide variations (from −1.1% to 1.3%) in each sample (see Table 2 in the online Data Supplement), possibly attributable to the close proximity of the retention times of HbVAR and HbA (mean 1.15 and 1.09 min, respectively), leading to inconsistent separation. Four immunoassays, the 2 boronate-affinity methods and 1 new enzymatic assay, were predicted to have good performance.

A few case studies of patients with Hb G-Coushatta have obtained results in accordance with the present data. One study of a Japanese Hb G-Coushatta family found that unexpectedly low HbA concentration results were obtained with the Daiichi Hi-AUTO A1c HA-8150 HbA1c analyzer (2.7%) than with the DCA2000 (4.7%) and electrospray mass spectrometry (5.0%) (16). Hb Queens is another common HbVAR in the Korean population. Hb Queens was eluted discretely on the 3 Bio-Rad instruments (VII, VII-T, and VII-TM) in the S-window. This HbVAR was also separated using G7-VM, HA8160-DM, and HA8160-TM, but was indistinguishable to 1.3% in each sample (see Table 2 in the online Data Supplement). This HbVAR was also separated using G7-VM, Bio-Rad instruments (VII, VII-T, and VII-TM) in the population. Hb Queens was eluted discretely on the 3

Table 1. Mean differences between the commercial methods and the IFCC PRM for samples containing HbVAR.

<table>
<thead>
<tr>
<th>Principle</th>
<th>Method</th>
<th>Hb G-Coushatta (n = 7)</th>
<th>Hb Queens (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6%</td>
<td>7%</td>
<td>6%</td>
</tr>
<tr>
<td>Boronate affinity</td>
<td>Ultra2</td>
<td>0.14</td>
<td>−0.08</td>
</tr>
<tr>
<td></td>
<td>PDQ³</td>
<td>0.09</td>
<td>−0.09</td>
</tr>
<tr>
<td>Ion-exchange HPLC</td>
<td>VII</td>
<td>−1.28a</td>
<td>−1.79a</td>
</tr>
<tr>
<td></td>
<td>VII-T</td>
<td>−1.49a</td>
<td>−1.99a</td>
</tr>
<tr>
<td></td>
<td>VII-TM</td>
<td>0.19</td>
<td>−0.07</td>
</tr>
<tr>
<td></td>
<td>G7−SM</td>
<td>−1.77a</td>
<td>−2.29a</td>
</tr>
<tr>
<td></td>
<td>G7−VM</td>
<td>−1.59a</td>
<td>−2.13a</td>
</tr>
<tr>
<td></td>
<td>HA8160−DM</td>
<td>−1.53a</td>
<td>−2.13a</td>
</tr>
<tr>
<td></td>
<td>HA8160−TM</td>
<td>−1.05a</td>
<td>−1.59a</td>
</tr>
<tr>
<td>Immunoassay</td>
<td>Unimate</td>
<td>0.05</td>
<td>−0.20</td>
</tr>
<tr>
<td></td>
<td>TinaQuan−II²</td>
<td>−0.03</td>
<td>−0.44</td>
</tr>
<tr>
<td></td>
<td>AU640</td>
<td>−0.42</td>
<td>−0.50</td>
</tr>
<tr>
<td></td>
<td>DCA2000</td>
<td>0.12</td>
<td>−0.15</td>
</tr>
<tr>
<td>Enzymatic assay</td>
<td>Norudia</td>
<td>0.00</td>
<td>−0.20</td>
</tr>
</tbody>
</table>

* Clinically significant difference (>0.6% at 6% and >0.7% at 7%).
³ n = 5 for Hb G-Coushatta, n = 9 for Hb Queens.

Comparison studies showed that the common HbVAR as well as some rare HbVAR in Koreans can interfere with the different HbA1c methods, particularly those performed on HPLC instruments with a short elution time. This interference can be resolved by measurement with unaffected methods such as HPLC with a long elution time, immunoassay, boronate affinity chromatography,
and enzymatic assay. The chromatograms of the ion-exchange HPLC methods also showed variable patterns, a finding that highlights the need for a careful inspection of the chromatograms, particularly for analysis of samples suspected of having an Hb\textsuperscript{VAR} or when an unexpected result is obtained. Overall, it is important to understand the effects of these Hb\textsuperscript{VAR} on the various methods.

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References


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