Incidence of Variant Hemoglobin (Hb) and Increased Fetal Hb Concentrations and Their Effect on Hb A1c Measurement in a Korean Population

To the Editor:

Glycohemoglobin assays are important for evaluating long-term glycemic control in patients with diabetes (1). Ion-exchange HPLC is commonly used for measuring hemoglobin A1c (Hb A1c), but hemoglobin variants (Hb\textsuperscript{var}) or increased fetal Hb concentrations may affect the quantification of Hb A1c by HPLC. The presence of Hb\textsuperscript{var} or high Hb F concentrations can be recognized by the separate elution of variant peaks or by abnormally high peaks on HPLC, and these abnormal findings arise mostly from genetic alterations in the globin genes (2, 3).

In Koreans, only limited data are available on the incidence and molecular genetic background of Hb\textsuperscript{var} and high Hb F concentrations. Therefore we investigated patients who had abnormal peaks on their Hb A1c analyses and characterized them by molecular methods.

Of the 27 006 patients who were tested for Hb A1c at Samsung Medical Center in Seoul, Korea, from May 2004 to October 2004, we investigated 25 patients who had abnormal results [variant peaks or high Hb F values (>5%)] on the Bio-Rad Variant II Turbo HPLC System. We carried out DNA studies of the globin genes by PCR and sequencing of all the coding exons, promoters, and introns of the β-, α1-, α2-, γ1-, and γ2-globin genes (4, 5). In the group with increased Hb F, we performed a gene dosage analysis on exon 1 of the β-globin gene with the LightCycler (Roche Diagnostics), with albumin coamplified as the reference gene.

Ten patients had variant peaks (Fig. 1). Among them, 5 patients had variant peaks between the A1c and A0 peaks (mean retention time, 0.73 min), which were identified as Hb G Coushatta (β\textsuperscript{22}Glu→Ala). One patient with variant peaks at the E, D window (retention time, 0.87 min)
had Hb Hoshida (β43Glu→Gln). All 4 patients with abnormal peaks at the S window on HPLC (mean retention time, 0.91 min) had Hb Queens (α1 34Leu→Arg). All 10 patients with variant peaks were heterozygous, and they had abnormal bands within the Hb S/Hb G/Hb D area on cellulose acetate Hb electrophoresis.

Hb G Coushatta has been found in Koreans, Chinese, and in some Japanese families (6). This variant usually leads to underestimation of Hb A_{2}, as we noted in our patients. The abnormal peak between Hb A_{2} and Hb A_{0} seemed to be that of glycated Hb G Coushatta, as Ogawa et al. (7) have indicated, and Hb G Coushatta is thought to coelute with the normal Hb A_{0} peak. Hb Hoshida has been reported in a few Japanese families and in 1 Yugoslavian family, and Hb Queens has been found in Koreans, Chinese, Japanese, and Vietnamese (6).

We found increased Hb F concentrations in 15 patients. Gene dosage analysis revealed that the ratios of the β-globin gene to the albumin gene were ~0.5 in 2 patients, which suggested heterozygous deletion of the β-globin gene. One of the 2 patients had no phenotypic abnormality other than the increased Hb F (19.5%), suggesting deleterious hereditary persistence of fetal Hb. The other patient had a history of chronic microcytic hypochromic anemia. The increased Hb A_{2} concentration, the decreased osmolality fragility, and the typical findings on the peripheral blood smear suggested deletional β-thalassemia minor. The remaining 13 patients showed negative results for all molecular analyses on the β- and γ-globin genes. Only the XmnI site sequence variation (C→158G→T) on the promoter of the γ-globin gene was noted in 10 patients, including 1 homozygote. This sequence variation has been shown to influence the Hb F concentrations in apparently healthy individuals (8). Hereditary persistence of fetal Hb was suspected because no phenotypic or laboratory abnormalities other than the increased Hb F concentrations were seen.

In conclusion, the incidences of Hb\textsuperscript{var} and high Hb F concentrations were estimated to be 1 in 2700 and 1 in 1800, respectively. The most common Hb\textsuperscript{var} in Koreans were Hb G Coushatta and Hb Queens, which could be presumed from their characteristic HPLC patterns. The known sequence variations in the β\textenvariants, G\textenvariants, or A\textenvariants that cause high Hb F are rare in Koreans. Considering that Hb\textsuperscript{var} and high Hb F concentrations are not uncommon, more effort should be made to estimate the correct Hb A_{2} value in Korean patients with diabetes.

References

Seung-Tae Lee
Myong Soo Kim
Dae Yong Choi
Sun Kyung Kim
Chang-Seok Ki

Department of Laboratory Medicine
Samsung Medical Center
Sungkyunkwan University
School of Medicine
Seoul, Korea

Preparation of a Chimeric Armored RNA as a Versatile Calibrator for Multiple Virus Assays

To the Editor:

As with all diagnostic techniques, molecular testing requires careful quality control (1–3). In detection of RNA viruses, which are often present at low concentrations and are prone to degradation, stringent monitoring is needed for all aspects of assay performance, including virus lysis, RNA isolation, reverse transcription, amplification, and detection steps. Among many proposed RNA control preparations (4, 5), armored RNA is currently the most suitable for clinical applications as it carries the viral RNA target of interest in a form that is ribonuclease-resistant, noninfectious, and stable after prolonged incubation in clinical matrices, and the preparations are substantially less expensive to manufacture than virus-infected plasma (6–8). Thus, armored RNA has been applied as a positive control for a variety of RNA viruses (9).

Because most commercial armored RNA preparations contain exogenous sequences of <500 nucleotides (9), separate armored RNA species are often prepared for calibration of each target in multiple virus assays. To reduce costs and simplify multi-virus detection, we are seeking to produce a single chimeric armored RNA species that might be used as a positive control for multiple viral targets. We consider this task to be feasible because the inventors of armored RNA predicted that, theoretically, at least 2 kb of nonbacteriophage RNA sequence might be encapsulated (8). As proof of this