mmol/L at 95% confidence from commonly estimated biochemical variables. We plan to see whether our method to calculate UFCa, which requires the concentrations of total Ca, alb, glob, and arterial blood pH, will provide a tool to give insights into the altered tubular handling of calcium in early renal failure.

References

A Nondiabetic Case of Hemoglobin Variant (Hb Niigata) with Inappropriately High and Low HbA1c Titers Detected by Different Methods, Tsuyoshi Watanabe, Ken Kato, Daishiro Yamada, Sanae Midorikawa, Wakano Sato, Masaru Shiga, Yoshihiko Otsuka, Masakazu Miura, Keiko Harano, and Teruo Harano

Human adult hemoglobin (Hb) consists of HbA (96% of the total), HbA2 (3%), and HbF (1%). HbA contains a number of subfractions, including HbA1a2, HbA1b, and HbA1c (glycosylated Hb). HbA1c has been used as a clinical marker for blood sugar control for the past one to two months. Inappropriately low or high HbA1c concentrations in comparison with blood glucose concentrations are caused by various conditions of Hb structure and metabolism; under such conditions, the use of HbA1c as a clinical marker cannot be warranted. Abnormally low HbA1c concentrations are usually encountered in patients with high turnover rates of Hb, whereas disproportionately high HbA1c concentrations are found under relatively rare conditions. Various hereditary Hb variants have been reported to cause disproportionately high and low HbA1c titers, as determined by chromatographic methods such as HPLC (1–6).

Recently, another simple and specific method for the quantitation of HbA1c, a latex immunoagglutination (LA) method that uses a monoclonal antibody against a glucose moiety at the β-chain N-terminal, has been developed and used in place of HPLC methods. However, the relevance of the LA method for measurement of HbA1c concentrations in cases with Hb variants has not been tested.

We report a case of a Hb variant (Hb Niigata) with inappropriately increased and decreased HbA1c measured with HPLC and LA, respectively. Thus, LA may have a limitation for the accurate quantitation of HbA1c in some special cases.

A 58-year-old male (weight, 58.8 kg; height, 162.5 cm) without any abnormal signs or symptoms was evaluated with routine laboratory data at the time of an annual health check provided by his employer. His HbA1c measured with LA was below the reference interval (2.9%), but his fasting blood glucose (FBG) was 5.4 mmol/L (98 mg/dL). His medical records over the preceding two years revealed HbA1c results by HPLC of 13.0% and

Fig. 1. Isoelectric focusing and sequencing gels showing presence of abnormal β-chain.

(A) Isoelectric focusing patterns (pH range, 6–9) of the hemolysates of the proband (lower lane) and a control subject (upper lane). (B) Photograph of a sequencing gel of the cloned cDNA encoding the β-globin gene of this case (left lane) and a control subject (right lane).
13.6% and FBG values of 6.0 and 6.2 mmol/L (108 and 111 mg/dL). His HbF and HbA2 titers were within reference intervals (<1% and 3.26%, respectively). His blood cell counts and biochemical parameters, including liver function tests, were unremarkable.

For the HPLC method, the column (TSK gel GlycoHS, Tosoh) was equilibrated with a solution containing, per liter, 12.2 mmol NaH₂PO₄, 2.8 mmol Na₂HPO₄, and 4 mmol KCN, pH 6.8. Samples were injected every 3.5 min. Elution was performed at a flow rate of 1.8 mL/min. The absorbance of the eluent was monitored at 450 nm, as well as at 690 nm as a reference wavelength, by a HPLC-723GHb automated analyzer (Tosoh).

For the LA method, latex beads were added to hemolyzed sample, and the hemoglobin and HbA1c nonspecifically adsorbed to the surface of the latex. A monoclonal antibody against a glucose moiety at the β-chain N-terminal was added to the mixture. Anti-mouse IgG goat antibody was then added to the reaction mixture to induce immunoagglutination of latex beads. The agglutination was monitored using an AU-600 automated analyzer (Olympus).

HbA1c values of healthy subjects (n = 30) determined by these HPLC and LA methods were 4.94 ± 0.08% and 4.82 ± 0.04% (mean ± SD), respectively. The values of HbA1c determined by HPLC were strongly correlated to those determined by the LA method (r = 0.917, n = 119).

Isoelectric focusing of the hemolysate was performed on a polyacrylamide gel plate containing carrier ampholyte (pH range 6–8 and 3.5–10.0; Pharmacia LKB Biotechnology).

HbF and HbA2 concentrations in the total Hb were quantified by HPLC on a DEAE-5PW column (7.5 × 75 mm, Tosoh); they were eluted with a linear NaCl gradient (0–0.5 mol/L) in 20 mmol/L Tris-HCl, pH 8.0. Detection and isolation of the abnormal β-globin chain was carried out on a urea-cellulose column (CM-52, Whatman Paper International) under chromatographic conditions described previously (8). The isolated β-globin chain was aminoethylated, and then digested with N-tosyl-phenylalanine chloromethyl ketone-trypsin. The resulting peptides were separated by reversed-phased HPLC (column, TSK gel ODS80Ts, 4.6 × 250 mm, Tosoh). The isolated abnormal peptide was hydrolyzed with constantly boiling HCl at 110 °C for 20 h. The amino acid composition was determined with an automatic amino acid analyzer.

RNA was obtained from peripheral blood using the QuickPrep mRNA Purification Kit (Pharmacia). β-globin cDNA was prepared using a cDNA Synthesis Kit (Pharmacia) and amplified by PCR using the GeneAmp amplification reagent kit (Perkin-Elmer Cetus). The reaction mixture, containing 0.1 μg of cDNA, 50 pmol of PCR primers, reaction buffer, dNTPs, and 0.1 of Taq polymerase, was placed in a Programmable Thermal Controller (MJ Research-Funakoshi) to produce the amplified DNA (9). The amplified DNA was ligated into the M13mp19 cloning vector, then transfected to the bacterium JM109 using the E. Coli Pulser (Nippon Bio-Rad Laboratories), and mixed single-stranded DNA (ssDNA) and cloned ssDNA were prepared (10). Nucleotide sequencing was performed by the dyeoxy method (Seque-
When eluted on HPLC, the patient’s hemolysate showed a single, symmetric band with an area of 13.1% of the total at an elution time corresponding to that of HbA1c (data not shown). Isoelectric focusing of the hemolysate detected an abnormal band between the HbA0 and HbF bands (Fig. 1A). Because of the width of this abnormal band, we speculated that it might be derived from β-globin. The molecular structure of the patient’s β-globin was analyzed as follows. The elution pattern of the digested peptides on reverse HPLC showed that the baT-1 peptide peak at the usual position diminished and a new peptide peak appeared near peptides βT-10 and βT-14. The amino acid composition of this abnormal peptide [Thr 1.03 (1), Glu 2.15 (2), Val 0.13 (1), Met 0.80 (0), Leu 1.93 (1), Lys 1.00 (1), His 0.98 (1), and Pro 1.00 (1)], where the number in parentheses shows the theoretical value of the peptide βT-1 indicates that in peptide βT-1, a valine residue at the β-1 position is replaced by a leucine residue and a methionine residue was added. Thus, this abnormal Hb appeared to be Hb Niigata [βN-methionyl-1(NA)Val→Leu].

To confirm this assumption, a mixture of cloned ssDNA templates was prepared and sequenced. The nucleotide sequence of the segment encoding peptide βT-1 was heterozygous; a G and a T were present at the first position of codon 1 (GTG-TTG); this agrees with the replacement of valine by leucine. The polymorphism of the third nucleotide of codon 2 was T on both the normal and abnormal β genes. Sequencing of the cloned ssDNA templates confirmed that this abnormal β-chain was Hb Niigata (Fig. 1B).

HbA1c used as a clinical marker for glycemic control in diabetic patients (11) can be measured by several methods, including colorimetry, electrophoresis, column chromatography, and immunooagglutination. The HPLC method has been used as a standard method for HbA1c determination. The LA method using a specific antibody has been used as a standard method for HbA1c determination. The LA method using a specific antibody has been used as a standard method for HbA1c determination. Hemoglobin 1992;10:11–7.

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