No Effect of Anticoagulant on Hb A1c Analysis by the IFCC Reference Procedure

To the Editor:

The use of glycohemoglobin (Hb A1c) as a diagnostic marker has been limited because of the lack of a standardized method. Hb A1c estimation with the commercially available instruments is based on different analytical and immunological principles that are method and reagent dependent. Consequently, several global initiatives have made steps toward optimizing and standardizing Hb A1c estimation. An IFCC working group carried out one such initiative, which is now considered the gold standard for Hb A1c measurement (1–4). This method is based on the proteolytic digestion of the N-terminal β chain of Hb A0 and Hb A00, followed by chromatographic separation of the hexapeptides and quantification by either liquid chromatography–mass spectrometry or capillary electrophoresis with ultraviolet detection.

Anticoagulants are used to prevent the coagulation of blood. The IFCC method uses EDTA as the anticoagulant. The effects of other anticoagulants have not been studied, however, although they are known to influence glucose analysis. We therefore analyzed the effect of the different anticoagulants (sodium heparin, lithium heparin, sodium fluoride, and sodium citrate) on Hb A1c measurement by the IFCC method.

Fresh blood samples were collected from 3 volunteers who had different Hb A1c percentages (low, medium, high) into Vacutainers (Becton Dickinson) containing different anticoagulants. Hemolysates were prepared and stored as per the IFCC method until analysis (1). Hemoglobin was estimated with the Beckman Coulter A1c-T diff™ cell counter calibrated by the method of the International Council for Standardization in Haematology (ICSH).

In accordance with the IFCC protocol, peptide linkages C-terminal to the glutamic acid residues in Hb components were cleaved with endoprotease Glu-C (Sigma-Aldrich) (1). The digested samples were quantified by liquid chromatography–mass spectrometry analysis with a microOTOF-Q mass spectrometer (Bruker Daltonics) linked to a liquid chromatography system from the Agilent 1200 series (Agilent Technologies). Val-His-Leu-Thr-Pro-Glu (VHLTP) nonglycated hexapeptide (Peptides International) and 1-deoxyfructosyl-VHLTP (tri-fluoroacetic acid salt) glycated hexapeptide (Peptide Institute) were used as calibrants. The deuterated counterparts [H-VHL(d10) TPE-OH] and 1-deoxyfructosyl-VHL(isopropyl d7)TPe were obtained from the above suppliers, respectively, and used as internal standards.

A calibration curve was constructed with 2%, 4%, 8%, 12%, and 14% glycated hexapeptide in a mixture of glycated and nonglycated hexapeptide calibrants (Rconc). The responses for each of the peaks at m/z 695.4, 705.5, 857.5, and 864.5 were obtained by integrating the peak areas. The signal from Hb A1c (Sx) was calculated from the ratio of the area of the peak at m/z 857.5 to the area of the peak at m/z 864.5. Similarly, the signal from Hb A0 (Sy) was calculated from the ratio of the area of the peak at m/z 695.4 to the area of the peak at m/z 705.5. Therefore, the ratio of the Hb A1c signal to the Hb A0 signal (Rsignal) was calculated from the Sx/Sy ratio. A calibration plot of Rsignal against Rconc was fitted to a linear model, and this plot was used to calculate the unknown Hb A1c concentrations in the hemolysates obtained from the volunteers. The Hb A1c percentages obtained for the different anticoagulants for volunteers with high, medium, and low glycemic index values were, respectively: EDTA, 9.07%, 6.13%, and 3.25%; lithium heparin, 8.57%, 6.16%, and 3.29%; sodium citrate, 8.71%, 5.98%, and 3.12%; sodium fluoride, 9.22%, 6.14%, and 3.13%; and sodium heparin, 9.32%, 6.10%, and 3.10%. CV values were 5%.

The Hb A1c percentages obtained in the study showed no variation with respect to the use of different anticoagulants. One of the reasons for this uniformity could be due to the lysis of erythrocytes before Hb A1c estimation. Moreover, the chromatographic separation of the digested products followed by their resolution as the m/z ratios of their protonated adducts [(M+H)+] is also independent of anticoagulants.

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References


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