The results (Table 1) were as follows: the slope (B1) was not detectably different from 1, and no nonlinearity (B2), reagent-to-reagent carryover (B3), or linear drift (B4) was detected (all conclusions based on statistical significance of P < 0.01).

This evaluation of the Abbott EPx clinical chemistry analyzer according to Krouwer et al. (1) showed that the instrument meets the criteria of good laboratory practice.

Reference

Novel Determination of Glycated Proteins in Biological Samples, Kunio Kobayashi, Koichi Yoshimoto, Kazumasu Hirouchi, and Kiyohisa Uchida (1 Biomed. Labs., and 2 Div. of Diagnostics, Shionogi & Co., Ltd., 2-5-1, Mishima, Settsu 566, Japan)

Measuring glycated proteins is diagnostically important in cases of diabetes mellitus with hyperglycemia (1, 2). Conventional methods for this have involved thiobarbituric acid (3), furoseine (4), affinity chromatography (5), and nitroblue tetrazolium (NBT)-reducing methods (6). However, these are complicated and time-consuming procedures or are influenced by the kind of protein present.

Here we tried measuring glycated protein by a novel method based on the colorimetry of 2-keto-glucose, which is released from the reaction of glycated protein (ketoamine) with hydrazine (7) (Figure 1). Briefly, a mixture of sample (0.1 mL) and an aqueous solution of hydrazine (4.0 mL, 0.1 mL) was heated at 100 °C for 0.5 h; we then added 0.6 mL of a 0.02 mol/L solution of phenylhydrazine in acetic acid (400 mL/L) and incubated this at 60 °C for 1 h. The absorbance of the supernate obtained after centrifuging (1700 x g, 10 min) the reaction mixture was measured at 390 nm.

The calibration curve for glycated human serum albumin (gHSA) showed a good linearity over the range of 0.70–1440 μmol/L. The lower detection limit for gHSA was estimated to be 0.70 μmol/L (fructosamine value). Intra-assay reproducibility (CV) was 4.4–10.9% at 11–453 μmol/L (n = 10). Analytical recovery of gHSA (3.6–720 μmol/L) added to pooled human serum was 87.4–97.9%. The concentration of glycated protein in clinical serum samples that had been preincubated with glucose oxidase (5 U) at 37 °C for 0.5 h and measured by the present method (y) correlated well with those (fructosamine value, x) measured by the NBT-reducing method: y = 1.2x – 7.1 (r = 0.92, n = 35).

The coloration increased linearly with time up to 10 min from the initiation of the reaction. After the coloration reached a plateau, its absorbance stayed constant at room temperature (25–26 °C) for ≥2 h. Thin-layer chromatographic analysis showed that N-p-tolyl-D-isoglucosamine (synthetic ketoamine) was completely converted into 2-keto-glucose, releasing p-toluidine upon heating with hydrazine. The subsequent quantitative reaction with phenylhydrazine gave D-glucose phenyllosazone (D-glucose-bis(phenylhydrazone); yellow pigment, λmax 389.8 nm in 1 mol/L acetic acid). The cross-reactivity of gHSA and N-p-tolyl-D-isoglucosamine was 102.6%, suggesting that the isoglucosamine was a useful calibrator for this assay system. D-Glucose, D-manucose, and D-galactose showed small reactivity (6.2–8.3%). The glucose-induced coloration was very slight; however, because the serum of a diabetic patient may contain 10–30 mmol of glucose per liter, such samples should be dialyzed or preincubated with glucose oxidase before assay to prevent false-positive results.

In conclusion, our method is novel, highly sensitive, and quick for measuring glycated proteins in biological samples.

References