Laboratory Assessment of a Commercial Kit for Measuring Fructosamine in Serum

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We have evaluated the laboratory performance and clinical usefulness of the Roche fructosamine kit. As used with an Abbott ABA 100 bichromatic analyzer, the kit response varied linearly with fructosamine concentration to 5.0 mmol/L (deoxymorpholinofructose equivalents). Interbatch precision was 4.1% and 3.6% for respective fructosamine concentrations of 3.2 and 5.0 mmol/L; intrabatch precision was 3.2% and 3.0% (fructosamine = 3.0 and 4.0 mmol/L). In 55 nondiabetic subjects all fructosamine values were <3.0 mmol/L, 95% were <2.7 mmol/L. For both fructosamine and glycated hemoglobin (Hb A₁c) the 95th percentile of the reference range corresponded to approximately the 10th percentile of values observed in 108 diabetic subjects. In the latter subjects fructosamine concentrations correlated somewhat (r = 0.6504) with the Hb A₁c value and for eight diabetic subjects indicated a similar degree of diabetic control over a 10-week period. From assessments of sensitivity and specificity for predicting abnormal glucose tolerance in 145 subjects, we conclude that this assay of serum fructosamine reflects diabetic control about as well as Hb A₁c estimation, but neither can replace the glucose tolerance test for the diagnosis of diabetes.

Additional Keyphrases: glycated hemoglobin and glucose tolerance test compared · diabetes · colorimetry · reference interval · cutoff values

The measurement of glycated hemoglobin (Hb A₁c) and serum albumin as indices of diabetic control is well established, yet the methods of measurement are often technically difficult, time consuming, and laborious (1). Recently, a manual colorimetric assay, based on the reducing activity of glycated serum proteins (fructosamine) has been reported (2–4), and a kit version of the method has been produced. Here we describe the results of laboratory and clinical studies undertaken to assess the performance and clinical usefulness of serum fructosamine measurements made with this commercial kit.

Materials and Methods

Analytical procedures: The serum concentration of fructosamine was determined by using the Roche (F. Hoffman–Le Roche & Co. Ltd., Basle, Switzerland) kit and an Abbott ABA 100 bichromatic analyzer (Abbott Laboratories, Pasadena, CA) set as follows: reaction direction, up; mode selector, rate; kinetic mode, normal; temp., 37 °C; analysis time, 5 min; no. of revolutions, 4; decimal point, 0.000; dilution plate, 1.11. The kit reagent was a carbonate buffer (0.1 mol/L, pH 10.35) containing 250 mmol of nitroblue tetrazolium chloride per liter. The fructosamine assay standard (3.55 mmol/L), used as supplied by Roche, was checked against dilutions of a 10 mmol/L laboratory standard prepared by weighing 1-deoxy-1-morpholinofructose (DMF; Sigma Chemical Co., St. Louis, MO) into a matrix of human serum albumin (40 g/L in isotonic saline) as described by Baker et al. (5).

Blood samples were drawn into plain Vacutainer Tubes (Becton Dickinson, Rutherford, NJ) at various times during the day; the serum was separated within 2 h of collection and stored at 6 °C. All fructosamine concentrations were determined within one week of collection.

Hb A₁c was determined by a liquid-chromatographic method previously described (6); we used chromatographic equipment from Waters Associates, Milford, MA. Glucose concentrations were determined with BM Test-Glycemia 20-800 strips and quantified with a Reflolux meter (Boehringer-Mannheim, Mannheim, F.R.G.) or by a glucose oxidase method (Glucinet; Sclavo Diagnostics, 20092 Cinisello Balsamo—MI, Italy).

Determination of fructosamine concentrations in diabetic...
and nondiabetic subjects: We measured fructosamine, Hb A1c, and random glucose concentrations in samples from 116 insulin-treated diabetic subjects routinely attending the diabetic clinic (62 men and 54 women, mean age 38 years). We determined a fructosamine reference interval from results for 55 healthy nondiabetic subjects (25 men and 30 women, mean age 35 years). The reference interval for Hb A1c was as reported previously (7).

Variability of fructosamine on repeated sampling: We assessed glycemic control in eight insulin-treated diabetic subjects over a 10-week period by measuring Hb A1c at weeks 1, 4, and 8 and comparing the results with the variation in weekly fructosamine concentrations determined over this period.

Relationship of fructosamine concentrations to glucose tolerance: Results for 146 subjects presenting for a standard 75-g oral glucose tolerance test (8) were grouped as indicating nondiabetic status, impaired glucose tolerance, or diabetic status. We then compared the glucose tolerance test results with the Hb A1c and fructosamine results for each group.

Results

Method Evaluation

Linearity: By using a commercially available quality-control serum serially diluted with a 40 g/L solution of human albumin in isotonic saline, we determined that the method gave results that varied linearly with fructosamine concentration to approximately 5.0 mmol/L, as assayed with the ABA 100 analyzer at the settings listed. Assay of the laboratory-prepared DMF standard showed similar linearity characteristics. We could increase the range of linearity to at least 10.0 mmol/L by using a more dilute serum (diluted 26-fold), but the fructosamine values obtained were somewhat higher whether the Roche standard or the laboratory-prepared DMF standard was used.

Precision: The interbatch precision (CV), obtained with commercially available quality-control serum, was 4.1% (n = 24, fructosamine = 3.2 mmol/L) and 3.6% (n = 16, fructosamine = 5.0 mmol/L). Similarly, the intrabatch precision was 3.2% (n = 10, fructosamine = 3.0 mmol/L) and 3.0% (n = 10, fructosamine = 4.0 mmol/L).

Storage: The fructosamine concentration in a serum stored for 15 days at 6 °C was 3.3 and 3.4 mmol/L on days 1 and 15, respectively.

Clinical Data

Normal reference interval: All fructosamine values in the 55 nondiabetic subjects were <3.0 mmol/L, the 95th percentile and median values being 2.7 and 2.3 mmol/L, respectively (Figure 1). Fructosamine concentrations correlated weakly with age (r = 0.3271, p < 0.01).

Fructosamine values in diabetic subjects: The distribution of fructosamine values measured in diabetic subjects routinely attending the diabetic clinic is also shown in Figure 1. These values ranged from 2.1 to 5.0 mmol/L (median 3.4 mmol/L), with 10% of the values being <2.7 mmol/L. By comparison, Hb A1c values in these subjects ranged from 4.5 to 16.5% (median 9.3%), and 7% of the values were <5.5% (the 95th percentile of the normal reference interval). The fructosamine concentration correlated significantly with the Hb A1c value (r = 0.6504, p < 0.001; Figure 2) and with random glucose measurements (r = 0.2933, p < 0.005; data not shown).

Variability on repeated samplings: Over the 10-week study Hb A1c measurements indicated stable glycemic control in seven of the eight subjects, while one subject appeared to have changing glycemic control. In this subject the Hb A1c was 10.5% of total hemoglobin at week 1 and 12.3% at week 8; the fructosamine concentration remained at 3.9 mmol/L for the first four weeks, then decreased to 3.2 mmol/L by week 10. The weekly fructosamine measurements indicated stable control in the remaining subjects, the CV of the weekly measurements for each subject ranging from 5.6 to 12.1%. The changes in the mean fructosamine and Hb A1c concentration for all subjects during the study period are depicted in Figure 3.

Oral glucose tolerance test: The fructosamine concentrations in the diabetic, impaired glucose tolerance, and nondiabetic groups, differentiated according to the WHO oral glucose tolerance test criteria (8), are shown in Figure 4. The median fructosamine concentrations of the diabetic (2.4 mmol/L) and nondiabetic (3.0 mmol/L) groups were significantly different (p < 0.001, Mann–Whitney U test) but there was marked overlap among the three groups, with abnormal fructosamine values detected in the nondiabetic group and normal values in the diabetic group. Fructosamine concentration did not discriminate the group with impaired glucose tolerance. There was a similar distribution of Hb A1c and fasting glucose values.

For the prediction of an abnormal glucose tolerance test result, a fructosamine measurement of >2.7 mmol/L, Hb A1c
Discussion

Several studies now support the determination of the serum fructosamine concentration as a quick and reproducible indication of the antecedent glycermic control in diabetic subjects (2-4). Here we have assessed a commercially available kit for the measurement of fructosamine.

Using an Abbott ABA 100 bichromatic analyzer, we found this method gave good inter- and intra-batch precision and linearity to 5.0 mmol/L. By comparison, similar precision but significantly greater linearity has been reported for use of a monochromatic analyzer (9). Linearity on the ABA 100 could be extended by using a greater dilution of the serum in reagent, but at the expense of some loss of accuracy. Presumably this was caused by a matrix interference at the secondary wavelength, which was not apparent at lesser dilutions. In our experience it is unusual to find fructosamine concentrations >5.0 mmol/L in diabetic subjects and we therefore find this degree of linearity acceptable.

Crystalline DMF is commercially available, but solutions of DMF in albumin and isotonic saline are not stable (even at -90 °C); thus the use of a secondary lyophilised standard as supplied in the kit is more satisfactory. We have found aliquots of the standard to be stable after reconstitution for as long as six months when stored at -90 °C.

The numerous different fructosamine reference ranges for nondiabetic individuals (2-4, 10) are presumably the result of problems with standardization, although the range we observed in the present study is in agreement with more recent publications (5, 9). We did note a correlation with age, but the variability was low, therefore making an age-adjusted normal range unnecessary. A similar finding has been reported for Hb A1c (11). Interestingly, we have found that the proportion of the diabetic population that falls within the normal range for fructosamine is the same as for the normal range for glycated hemoglobin.

We have observed a statistically significant correlation of serum fructosamine concentration with glycated hemoglobin that is in accord with previous publications (9, 12), but some individual observations are clearly different. Close agreement should not be expected, however, because the two measurements reflect integration of blood glucose values over different time intervals: one to two weeks for fructosamine (4) and up to eight weeks for glycated hemoglobin (13). Similarly, the agreement between fructosamine and Hb A1c as indicators of glycermic control over a 10-week period in eight insulin-dependent diabetics further supports the contention of previous authors that serum fructosamine may be used interchangeably with Hb A1c (4, 10). In the subject with changing glycermic control, the fructosamine concentration would probably have more closely reflected the glucose concentration.

The median concentration of fructosamine in serum from diabetic subjects, in a group of subjects presenting for oral glucose tolerance testing, was significantly different from that for the nondiabetes of the group. There was, however, considerable overlap between the two groups such that the sensitivity of the fructosamine to detect a diabetic glucose tolerance curve was 72%; the corresponding sensitivity of Hb A1c was 78%. Other workers, with fewer subjects, have reported slightly higher sensitivities (12, 14), but in a study with 333 outpatients, Coignet and Thiburt (15) reported a sensitivity of 60% for Hb A1c, with reference to the 2-h plasma glucose test. Unlike previous authors (12, 14), we believe that the concentration of fructosamine (or Hb A1c, for that matter) in serum is insufficiently sensitive to be used as a test for glucose intolerance.

Glycated hemoglobin is now widely used as an aid to determine the effectiveness of therapy for glycermic control...
in diabetic patients. In the present study, we have found that a measurement of serum fructosamine would give similar information as an Hb A1c estimation. Although some physicians may prefer the longer integration of blood glucose concentrations offered by measurement of Hb A1c, we find that measuring fructosamine has some advantage, the assay being technically simple and easily automated. Indeed, the kit assessed here gave a precise and reliable quantification of fructosamine concentration in serum.

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References

Rate Nephelometry and Radial Immunodiffusion Compared for Measuring Serum Prealbumin

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We compared a rate-nephelometric method and a radial immunodiffusion (RID) assay for measurement of prealbumin (transthyretin) in 55 samples of serum from healthy children. The mean prealbumin concentration as measured by the Beckman Auto ICS nephelometer was 188 mg/L (range 128–350); the mean by RID was 221 mg/L (range 125–419). This difference was statistically significant by Student’s t-test (p <0.05), but the correlation coefficient (r) was 0.95. To determine a reference interval for prealbumin in children by the Auto ICS method, we assayed samples from 93 healthy children between the ages of one day and 18 years (55 boys, 38 girls). The mean was 191 mg/L, the reference interval (mean ± 2 SD) 109–273 mg/L. There was no significant difference in prealbumin concentrations between girls and boys (Student’s t-test, p >0.05). Evidently the Beckman Auto ICS method measures prealbumin in serum rapidly and accurately.

Additional Keyphrases: pediatric chemistry · reference interval · nutritional status · transthyretin

In light of estimates that up to half of hospitalized patients may be malnourished (1,2), assessment of protein-energy status is becoming an increasingly important component of medical practice. Treatment by enteral and parenteral routes have improved the nutritional status of patients, but there continues to be a need more accurately to monitor patients receiving therapy. Nutritional assessment of patients has included anthropometric, biochemical, and clinical measurements and dietary control (7). Biochemical assessments have consisted of measuring albumin and transferrin in serum; however, the relatively long biological half-lives of these analytes (20 and nine days, respectively) limit their usefulness (3–5). In the last few years, prealbumin has received increasing attention in the literature as a potentially better nutritional marker with a shorter biological half-life (3, 6–7).

Prealbumin, also known as transthyretin or thyroxin-binding prealbumin, is a 54 000-Da glycoprotein synthesized in the liver; its half-life is two days. It is a carrier of retinol-binding protein and transports about a third of the thyroxin in blood (7). Radial immunodiffusion (RID) is the method most often

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