Specific Affinity-Chromatographic Measurement of Glycated Hemoglobins in Uremic Patients

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The clinical utility of glycated hemoglobin measurements in renal failure is controversial, given numerous earlier studies showing no correlation between glycated hemoglobin and other indicators of blood glucose control in uremic subjects. This problem is attributable, in part, to analytical interferences from carbamylated hemoglobins. We report use of a specific affinity method to measure glycated hemoglobins in a group of uremic patients, diabetic and nondiabetic, undergoing treatment by continuous ambulatory peritoneal dialysis. Concentrations of glycated hemoglobins correlated significantly with values for fasting plasma glucose (r = 0.52, n = 17, p < 0.05) in these patients and with mean glucose measurements in five diabetic patients who used home glucose monitoring (r = 0.91, p < 0.05). Contrary to studies with ion-exchange chromatography, our measurements of glycated hemoglobin showed no positive correlation with concentrations of urea in serum. In a separate group of patients, we found that hemodialysis sessions produced no acute effect on glycated hemoglobin. Measurements of glycated hemoglobins by analytically specific methods may thus better reflect long-term control of blood glucose in renal dialysis.

**Additional Keyphrases:** diabetes · end-stage renal failure · glucose · carbamylated hemoglobin · renal dialysis

Measurement of glycated hemoglobins in blood is generally accepted as a useful indicator of diabetic control (1), the concentrations of hemoglobin A₁c or of "fast" hemoglobin (A₁) having been shown to correlate well with the mean concentration of glucose in plasma and with other indicators of time-averaged glucose concentration in nonuremic diabetic patients (2). In uremic patients, however, reports on the usefulness of these measurements have drawn conflicting conclusions (3-16). With few exceptions (4, 14), previous studies have suggested that the concentration of glycated hemoglobins was increased in uremic patients (3, 9, 11-14, 16) and correlated with plasma concentrations of urea (7, 9, 16) or creatinine (5, 8, 9) but not with glucose (5, 6, 8, 11, 15, 16). These conclusions have been based on studies of patients being treated with continuous ambulatory peritoneal dialysis (10, 12, 13), hemodialysis (3, 6-9, 11, 13, 15, 16), or conservative therapy (5, 6, 7, 9, 11, 16). The discrepancies among these reports are apparently related at least in part, to interference in the assays from other altered hemoglobins in uremic blood (7). In particular, carbamylated hemoglobin, apparently formed from reaction with urea-derived cyanate ions (7), has much the same net electrical charge as some glycated hemoglobins and cannot be separated from them by analytical methods based on ion-exchange chromatography (7).

An affinity-chromatographic assay for glycated hemoglobin described recently by Mallia et al. (17) does not depend on the electrical charge of the molecule, but rather makes use of the binding of the glucose residues in the glycated hemoglobins to aminophenylboronic acid coupled to a carbonyl diimidazole-activated agarose matrix (6). Carbamylated hemoglobin will not affect the results because the carbamyl group does not contain the cis-dirol configuration that is recognized by the affinity method.

We evaluated this method in a group of patients with end-stage renal failure, who were being treated with continuous ambulatory peritoneal dialysis. These patients were particularly interesting because a high proportion of them were diabetics (47%) and because they were exposed to a high glucose concentration (from 15 to 45 g/L) in the dialysate. In addition, such patients tend to have higher concentrations of urea in their blood than do hemodialysis patients and thus may attain very high blood concentrations of carbamylated hemoglobins.

**Materials and Methods**

Blood samples were collected from all of the 17 patients (nine men and eight women, fasting and nonfasting) receiving continuous ambulatory peritoneal dialysis treatment at the University of Virginia Medical Center during the two-month period of study. Eight of the patients were diabetics, six insulin-dependent, and two noninsulin-dependent. One nondiabetic patient was obese and had nonfasting values for plasma glucose consistently exceeding 1000 mg/L. Plasma glucose values during fasting were consistently less than 1100 mg/L in the other eight nondiabetic patients. The mean age of the eight diabetic patients was 50 years (range 23-64), not significantly different from the mean age of the eight nonobese, nondiabetic patients (mean age 53 years, range 28-80).

We measured glycated hemoglobin by our modification (18) of the method of Mallia et al. (17). In brief, we hemolyze erythrocytes in 20 volumes of de-ionized water and separate the glycated hemoglobins in 100 μL of hemolyzate by affinity chromatography on a 1-mL column of m-aminophenylboronic acid immobilized on a support of cross-linked agarose. We elute the nonglycated hemoglobins with 20 mL of acetate buffer (250 mol/L, pH 8.0), then elute the glycated hemoglobins with 5 mL of buffer containing sorbitol, 300 mmol/L. To determine hemoglobin concentrations in the eluate, we measure absorbance at 414 nm with a Model 35 spectrophotometer (Beckman Instruments, Brea, CA 92621). The reference integral for the proportion of glycated hemoglobin by this assay, based on measurements in 143 nondiabetic volunteers (18), is 4.8% to 6.4% [(glycated/total) × 100%]. The day-to-day imprecision (SD) is less than 0.3% (n = 20) (18).

We used an Astra analyzer (Beckman Instruments) to

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measure serum urea (urease method) and glucose (glucose oxidase method, with oxygen electrode). Microhematocrits were measured by centrifugation.

We also collected paired pre- and post-dialysis blood samples from nine additional patients who were undergoing intermittent hemodialysis treatment, to assess the acute effect of hemodialysis on the concentration of glycated hemoglobin.

Results

The mean (±SE) proportion of glycated hemoglobin as measured by the affinity method was significantly higher in the diabetics (9.5% ± 1.1%) than in the nondiabetics (5.6% ± 0.2%) (Figure 1, p < 0.005 by unpaired t-test with unequal variances). Glycated hemoglobin was not increased in any of the eight nondiabetic patients but was increased in seven of the eight diabetic patients (p < 0.005, chi-square test) and in the obese patient (Figure 1). The one diabetic patient whose glycated hemoglobin was not increased was using home monitoring of glucose; her mean concentration of plasma glucose was 790 mg/L (see below), and she had a history of frequent episodes of hypoglycemia.

Glycated hemoglobin concentration correlated significantly (r = 0.52, p < 0.05) with fasting concentrations of plasma glucose (Figure 2, top). Five of the diabetic patients monitored their glucose at home and recorded at least three glucose values at various times each day (fasting or nonfasting not specified). The mean glucose concentration for these patients, computed from the measurements of glycated hemoglobin over the preceding month, was closely reflected by the values for glycated hemoglobin (r = 0.91, p < 0.05) (Figure 2, middle).

Values for glycated hemoglobin correlated negatively with the concentrations of urea nitrogen (Figure 2, bottom) (r = 0.52, p < 0.05). The negative correlation between these two variables was similar within the diabetic group of patients considered alone (r = −0.59) and within the group of nondiabetic patients alone (r = −0.55), although neither of these correlations was statistically significant (p > 0.1).

In contrast to a previous report by Dandona et al. (4), glycated hemoglobin was not significantly correlated with hematocrit of the peritoneal dialysis patients (r = 0.12, p > 0.10, n = 17). Moreover, results by the current assay were linearly related to sample dilution (18)—unlike the assay used by Dandona et al., which they (4) and others (19) found yielded artifically low results from samples with low concentrations of hemoglobin.

In view of a previous report of an increase in glycated hemoglobin after hemodialysis (20), we measured glycated hemoglobin in paired pre- and post-dialysis blood samples from nine additional patients undergoing hemodialysis. The proportion of glycated hemoglobin was not significantly changed in the post-dialysis samples (mean change = −0.26%, p > 0.10).

Discussion

In contrast to several previous reports (5, 6, 8, 11, 13, 15, 16), we have found that the proportion of glycated hemoglobin does reflect control of plasma glucose in uremic patients (Figures 1 and 2).

The discrepancy between our results and those in most previous reports was not unexpected, given the findings of Fluckiger et al. (7), who demonstrated that the positive correlation between blood urea and hemoglobin A1 did not persist when glycated hemoglobin was measured by the thiobarbituric acid method. Fluckiger et al. reported that at least part of the apparent increase in glycated hemoglobin
in uremia was caused by carbamylated hemoglobins formed from the condensation of urea-derived cyanate with N-terminal amino acids of hemoglobin (7). Their results and ours therefore suggest that conclusions of earlier reports do not apply to measurements of glycated hemoglobins by the more specific methods involving thiobarbituric acid or boronate affinity chromatography.

Interpretation of the glycated hemoglobin values, however, requires consideration of the shortened survival time of erythrocytes in uremia, the degree of glycation of hemoglobin being dependent on the age of the erythrocytes and thus on how long their hemoglobin was exposed to the circulating plasma glucose (1, 2). Interestingly, the proportion of glycated hemoglobin was not increased in most of the nondiabetic patients we studied, despite the well-known carbohydrate intolerance of uremic patients (21); decreased erythrocyte survival in these patients may have masked some degree of carbohydrate intolerance. The negative correlation of glycated hemoglobin with concentrations of serum urea (Figure 2, bottom) may reflect a greatly shortened erythrocyte survival in those patients with the highest concentrations of serum urea, in line with the studies of Shaw (22), who showed that erythrocyte survival was inversely related to the concentration of serum urea. Thus, the use of glycated hemoglobin for monitoring changes in diabetic control in a uremic individual clearly depends on a reasonable constancy of the patient's erythrocyte survival time during the period of study. Other factors influencing the formation of the glycated hemoglobin include oxygen tension, 2,3-diphosphoglycerate concentration (23), and even a potential membrane factor (24); the impact of uremia on the effect of these and other factors (including carbamylation) remains to be determined. We conclude from these results, however, that measurements of glycated hemoglobin by analytically specific methods can accurately reflect plasma glucose values in uremic patients, and that the results are not affected acutely by hemodialysis sessions.

References