Comparison of glycated albumin and hemoglobin A_{1c} levels in diabetic subjects on hemodialysis

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Glycated albumin is thought to more accurately reflect glycemic control in diabetic hemodialysis patients than hemoglobin A_{1c} because of shortened red cell survival. To test this, glycated hemoglobin and albumin levels were measured in blood samples collected from 307 diabetic subjects of whom 258 were on hemodialysis and 49 were without overt renal disease. In diabetic subjects with renal disease, relative to those without, the mean serum glucose and glycated albumin concentrations were significantly higher while hemoglobin A_{1c} tended to be lower. The glycated albumin to hemoglobin A_{1c} ratio was significantly increased in dialysis patients compared with the controls. Hemoglobin A_{1c} was positively associated with hemoglobin and negatively associated with the erythropoietin dose in hemodialysis patients, whereas these factors and serum albumin did not significantly impact glycated albumin levels. Using best-fit multivariate models, dialysis status significantly impacted hemoglobin A_{1c} levels without a significant effect on glycated albumin. Our results show that in diabetic hemodialysis patients, hemoglobin A_{1c} levels significantly underestimate glycemic control while those of glycated albumin more accurately reflect this control.

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The vascular complications of diabetes mellitus, including kidney disease, heart attack, and stroke are increasing rapidly throughout the world.[1] Diabetic nephropathy now accounts for nearly 50% of incident dialysis patients in the United States, and cardiovascular disease is the leading cause of death.[2] It is important to control conventional atherosclerotic risk factors in diabetic dialysis patients, including blood glucose, hypertension, hyperlipidemia, and smoking to reduce cardiovascular disease events.

Several clinical tests are useful for measuring long-term glycemic control in the general diabetic population. These same tests are routinely performed in diabetic subjects with chronic kidney disease and end-stage renal disease (ESRD); however, their accuracy in these patients has not been rigorously tested.[3] Hemoglobin A_{1c} (HbA_{1c}), the most widely used assay, measures the percentage of circulating hemoglobin that has chemically reacted with glucose and reflects ambient blood glucose control over the prior 120 days, with the most profound effect in the preceding 30 days.[3,4] Factors that shorten red blood cell (RBC) survival, including severe nephropathy, may reduce HbA_{1c} since the time necessary for glucose to chemically bond with RBCs decreases.[5] If this significantly impacts HbA_{1c}, dialysis patients and clinicians would be falsely comforted by relatively low HbA_{1c} values despite high risk for subsequent cardiovascular disease and infectious complications. Previous studies attempting to address this concern were underpowered.[6,7]

Inaba *et al.*[8] determined that HbA_{1c} underestimated long-term glycemic control in dialysis patients with diabetes, after comparing the mean of random blood glucose concentrations, HbA_{1c}, and percentage of glycated albumin (% GA). They found that the % GA assay provided a more accurate assessment of glycemic control among Japanese hemodialysis patients. The current study attempted to validate this clinically important result and extend its application to other ethnic groups.

RESULTS

Simultaneous blood samples were collected from 307 diabetic patients, 258 were receiving chronic hemodialysis treatments

Table 1 | Demographic and clinical characteristics of study population

Variable	Diabetic ESRD (*N*=258)	Diabetes, no nephropathy (*N*=49)	*P*-value
Age, years	63.2 (12.0)	59.1 (13.8)	0.060
DM duration, years	19.1 (10.8)	11.8 (8.4)	<0.0001
% male	53.1	40.8	0.115
% African American	63.6	32.7	<0.001
Height, inch	67.3 (4.0)	67.3 (3.8)	0.93
Weight, lb	189.7 (48.8)	237.3 (63.7)	0.007
% DM type 2	93.8	93.9	0.983
% ACEi/ARB	50.0	81.4	0.001
% current/former smokers	43.2	47.9	0.545
% transfused prior 90 days	7.0	0	0.089
Serum creatinine, mg per 100 ml	NA	1.0 (0.3)	—
Serum glucose, mg per 100 ml	172 (62)	146 (66)	0.024
Glycated albumin, g per 100 ml	0.69 (0.28)	0.62 (0.24)	0.071
Serum albumin, g per 100 ml	3.7 (0.4)	4.0 (0.6)	0.012
% glycated albumin	18.7 (7.3)	15.3 (5.5)	0.002
Hemoglobin, g per 100 ml	11.8 (1.1)	12.7 (1.8)	0.058
HbA_{1c}, %	6.8 (1.6)	7.3 (1.4)	0.058
% GA/HbA_{1c}	2.72 (0.6)	2.07 (0.5)	0.0001
Erythropoietin dose, U week^{-1}	22 876 (20 579)	NA	—

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; DM, diabetes mellitus; ESRD, end-stage renal disease; HbA_{1c}, hemoglobin A_{1c}; NA, not applicable; % GA, percentage of glycated albumin.

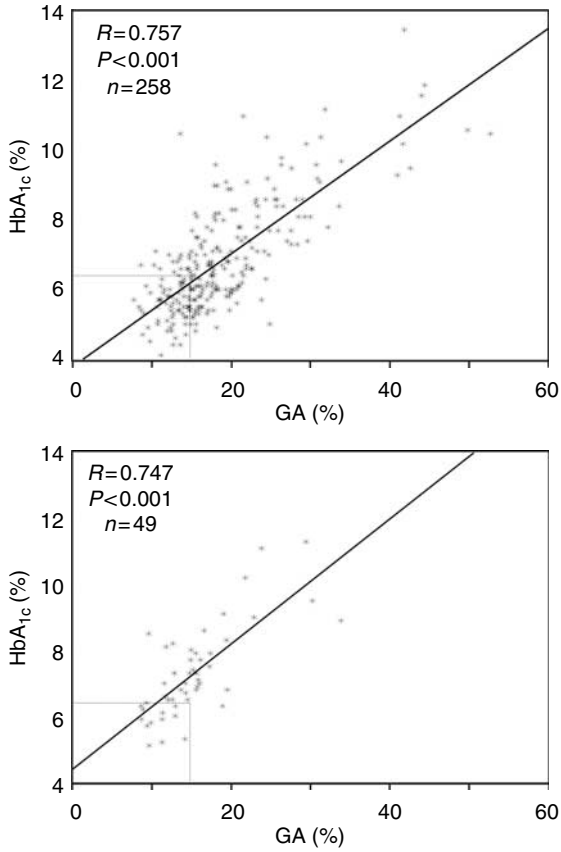

Figure 1 | Correlation between HbA_{1c} and glycated albumin, by ESRD status. Relationship between % HbA_{1c} and % GA in diabetic subjects with ESRD (upper panel) and without nephropathy (lower panel). Slopes of the lines differ between groups (0.163 for diabetes mellitus (DM)-ESRD, 0.190 for DM nonnephropathy patients, $P=0.0001$). Reference lines represent normal values.

and erythropoietin for advanced chronic kidney disease (ESRD), and 49 were without kidney failure. Table 1 contains demographic characteristics of the study population. All patients with ESRD received erythropoietin, with mean average (s.d.) weekly dose 22 876 (20 579) units. In all, 7% (18/258) of dialysis patients and 0% of those without kidney failure received a blood transfusion within the 90 days preceding the study. Subjects without kidney failure had mean serum creatinine concentration 1.0 (0.3) mg per 100 ml and ESRD patients had mean urea reduction ratio 70.9 (8.8)%, K_t/V 1.5 (0.4), hemoglobin 11.8 (1.1) g per 100 ml, transferrin saturation 25.4 (10.8)%, and ferritin 567 (424) ng ml^{-1}. Overall, the mean (s.d.) serum glucose concentration in all participants was 168 (63) mg per 100 ml. The nonnephropathy group had a mean (s.d.) serum glucose concentration of 146 (66) mg per 100 ml and in ESRD subjects 172 (62) mg per 100 ml. The coefficient of variation was 100*(63/168) or 37.5%.

In ESRD patients, relative to those without kidney disease, mean (s.d.) serum glucose concentrations were higher (172 (62) vs 146 mg per 100 ml (66); $P=0.024$), % GA was higher (18.7% (7.3), range: 7.7–52.7 vs 15.3% (5.5), range: 8.6–33.8%; $P<0.0001$), and HBA_{1c} was lower (mean (s.d.), range: 6.8% (1.6), 4.1–13.5 vs 7.3% (1.4), 5.1–11.3%; $P=0.058$) (Table 1). The % GA/HbA_{1c} ratio was significantly higher in ESRD patients relative to those without nephropathy (2.72 (0.6) vs 2.07 (0.5); $P=0.0001$). The % GA/HbA_{1c} ratio in diabetic subjects with ESRD compared with those without kidney disease should approximate 1, if these assays performed equally in both groups. Instead, the % GA/HbA_{1c} ratio in dialysis patients compared with subjects without nephropathy was 1.31 (2.72/2.07).

Figure 1 displays the correlation between HbA_{1c} and % GA in hemodialysis patients and subjects without kidney disease. A significant difference in the slope of the line expressing this relationship among hemodialysis patients (slope = 0.163) and subjects without nephropathy (slope = 0.190) was observed ($P=0.0001$). Figure 2 displays HbA_{1c} and % GA correlations with recent random blood glucose measures. No significant differences in the relationship between % GA and glucose concentrations were observed between participants on hemodialysis and those without kidney disease ($P=0.19$), whereas marked differences were observed with HbA_{1c} and glucose concentrations ($P<0.0001$).

Table 2 contains the results of subgroup analyses. In analyses restricted to African Americans, % GA/HbA_{1c} ratios were 2.77 in subjects with ESRD and 1.98 in those with normal kidney function ($P=0.0001$). Among non-African Americans (predominantly Caucasians), the ratios were 2.62 in those with ESRD and 2.12 in those without kidney disease

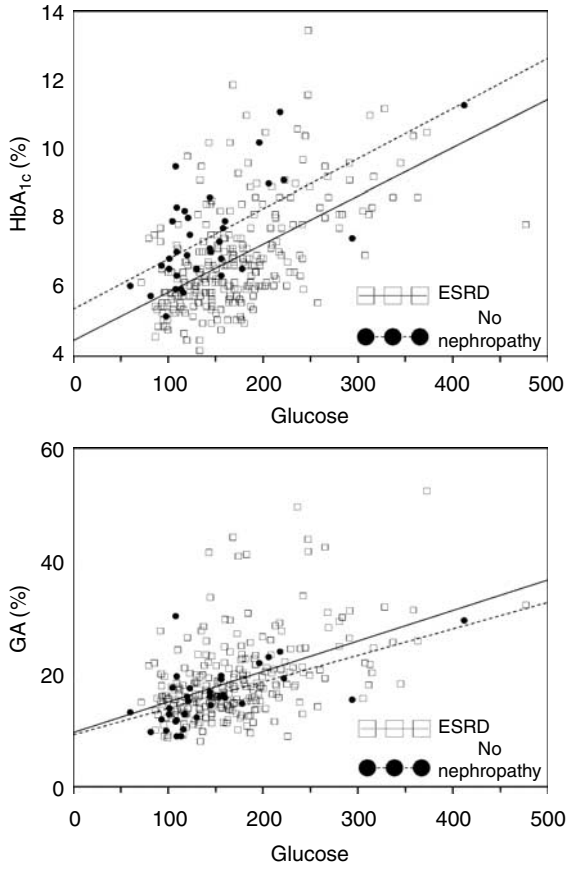

Figure 2 | Correlation between HbA_{1c} and glycated albumin assays with random serum glucose concentrations, by ESRD status. Upper panel: HbA_{1c} vs mean serum glucose concentration; when testing the regression lines there is a significant difference between diabetic hemodialysis patients and those without nephropathy, $P<0.0001$. Lower panel: % GA vs mean serum glucose concentration; testing the regression lines there is no significant difference between the groups, $P=0.19$. Circles depict subjects without nephropathy; boxes depict dialysis patients.

($P=0.001$). The % GA/HbA_{1c} ratios were significantly different in African American and non-African American ESRD patients ($P=0.049$) but not in African American and non-African American subjects without nephropathy, respectively. Relatively few African American subjects without nephropathy were evaluated.

Multivariate linear regression was then performed to determine which factors were associated with HbA_{1c} (Table 3). In a reduced best-fit model, serum glucose concentration ($P<0.0001$), age ($P<0.0001$), and dialysis status ($P<0.0001$) were all significant predictors of HbA_{1c}. A similar regression analysis was performed with % GA as the outcome variable. In a reduced best-fit model, serum glucose concentration ($P<0.0001$), age ($P=0.006$), gender ($P=0.002$), ethnicity ($P=0.021$), and diabetes duration ($P=0.001$) were significant predictors of % GA, while dialysis status was not.

As the % GA/HbA_{1c} ratio was significantly different in patients with ESRD and those with normal kidney function, a multivariate model adjusting for age, gender, ethnicity, and diabetes duration was fit to control for those variables. This model revealed similar results; as the % GA/HbA_{1c} ratio remained significantly different in diabetic subjects with ESRD and without kidney disease ($P=0.003$), with gender ($P=0.003$), serum glucose concentration ($P=0.001$), ethnicity ($P=0.048$), and diabetes duration ($P=0.001$) also significantly related. Age was not associated ($P=0.41$) with this outcome.

Further analyses were conducted in diabetic subjects with ESRD on hemodialysis. Figure 3 depicts the relationship between HbA_{1c} with both hemoglobin concentration and erythropoietin dose. Hemoglobin concentration and HbA_{1c} were positively associated ($P=0.02$); while erythropoietin dose and HbA_{1c} were negatively associated ($P=0.04$). Figure 4 reveals that the % GA was not significantly associated with serum albumin, hemoglobin concentration, or dose of erythropoietin.

Table 2 | Subgroup comparisons, by ethnicity and affection status

Ethnicity	Group	N	GA, %	HbA_{1c}, %	Ratio
African American	DM-ESRD	164	19.3 ± 7.5	6.9 ± 1.6	2.77 ± 0.66
African American	DM no nephropathy	16	13.9 ± 3.7	7.0 ± 1.2	1.98 ± 0.40
P-value			0.0007	0.80	0.0001
Non-African American	DM-ESRD	94	17.7 ± 6.8	6.7 ± 1.5	2.61 ± 0.59
Non-African American	DM no nephropathy	33	16.0 ± 6.1	7.4 ± 1.5	2.12 ± 0.52
P-value			0.21	0.01	0.001
Group	**Ethnicity**	**N**	**GA, %**	**HbA_{1c}, %**	**Ratio**
DM-ESRD	African American	164	19.3 ± 7.5	6.9 ± 1.6	2.77 ± 0.66
DM-ESRD	Non-African American	94	17.7 ± 6.8	6.7 ± 1.5	2.61 ± 0.59
P-value			0.083	0.27	0.049
DM no nephropathy	African American	16	13.9 ± 3.7	7.0 ± 1.2	1.98 ± 0.40
DM no nephropathy	Non-African American	33	16.0 ± 6.1	7.4 ± 1.5	2.12 ± 0.52
P-value			0.30	0.32	0.42

DM, diabetes mellitus; ESRD, end-stage renal disease; GA, glycated albumin; HbA_{1c}, hemoglobin A_{1c}.

Table 3 | Results of multivariate analyses in diabetic subjects with ESRD

Variable	HbA_{1c} full model *P*-value	HbA_{1c} reduced model *P*-value	% GA full model *P*-value	% GA reduced model *P*-value
Age	<0.0001	<0.0001	0.004	0.006
Ethnicity	0.12	0.08	0.028	0.021
Gender	0.11	0.09	0.001	0.002
Diabetes duration	0.096	0.051	0.006	0.001
Serum glucose	<0.0001	<0.0001	<0.0001	<0.0001
ACEi/ARB	0.91	NS	0.43	NS
Smoking status	0.57	NS	0.088	NS
Albumin	0.67	NS	0.81	NS
Dialysis status	0.0002	<0.0001	0.75	0.83

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; DM, diabetes mellitus; ESRD, end-stage renal disease; HbA_{1c}, hemoglobin A_{1c}; NS, not significant; % GA, percentage of glycated albumin.

Finally, a best-fit multivariate model was constructed for hemodialysis patients including all laboratory parameters (data not shown). Predictors of the HbA_{1c} included mean random serum glucose concentration ($P<0.0001$), age ($P<0.0001$), erythropoietin dose ($P=0.012$), number of years on dialysis ($P=0.008$), and diabetes duration ($P=0.04$). A similar model was constructed for % GA; where significant values included mean random serum glucose concentration ($P<0.0001$), age ($P<0.0001$), body mass index ($P<0.0001$), and diabetes duration ($P=0.046$).

DISCUSSION

Accurate determination of glycemic control is of paramount importance in the diabetic population, as improved glycemic control reduces micro- and macrovascular complications in patients with type 1 and type 2 diabetes mellitus.[9,10] Diabetic dialysis patients are especially at high risk, as more than 23% of incident diabetic dialysis patients in the United States will succumb to cardiovascular and infectious complications during their first year of renal replacement therapy and only 31% survive 5 years.[2] Glycemic control improves survival in patients on hemodialysis.[11,12] Moreover, mild degrees of hyperglycemia in nondiabetic dialysis populations have been associated with reduced survival.[13] An accurate assessment of glycemic control in the dialysis population is therefore critical, to improve outcomes and survival.

HbA_{1c} has been a cornerstone in the evaluation of dialyzed and nondialyzed diabetic patients. This measurement relies on a relatively stable RBC survival, a characteristic typical of the general population but not patients on hemodialysis. During hemodialysis, the uremic environment, blood loss during treatments, and frequent phlebotomy all contribute to decreased RBC lifespan. Shortened RBC survival and red cell transfusions are likely to lower the HbA_{1c}, potentially making it unreliable in assessing glycemic control. Indeed, in a large study that ultimately detected a relationship between HbA_{1c} and dialytic survival, a significant relationship was not detected until controlling for differences in the hemoglobin concentration.[12] The current investigation provides further insights into factors that are associated with HbA_{1c} values.

This report demonstrates that HbA_{1c}, relative to % GA, significantly underestimates glycemic control in diabetic dialysis patients. In those with ESRD, lower HbA_{1c} values were also associated with lower hemoglobin concentration and higher doses of erythropoietin. The treatment of chronic kidney disease-associated anemia was revolutionized with erythropoietin, a hormone that stimulates RBC formation and slightly increases RBC survival.[14,15] Dialysis patients with lower hemoglobin concentrations and receiving high doses of erythropoietin may have reduced RBC survival and shorter hemoglobin lifespan that is not overcome with high doses of erythropoietin. In contrast, % GA was not associated with hemoglobin, serum albumin concentration, or erythropoietin

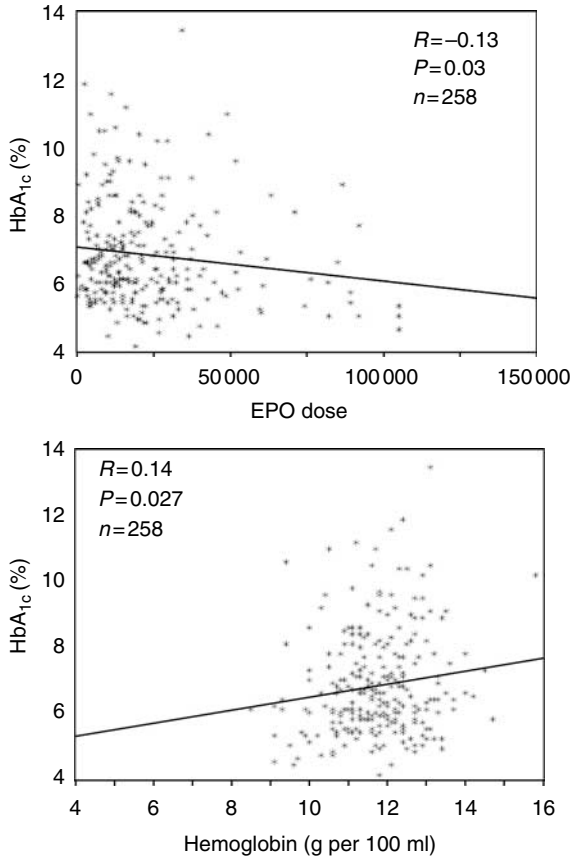

Figure 3 | Impact of hemoglobin concentration and EPO dose on HbA_{1c} in diabetic ESRD. Relationship between HbA_{1c} and weekly erythropoietin dose (upper panel); and hemoglobin concentration (lower panel) in diabetic subjects on hemodialysis.

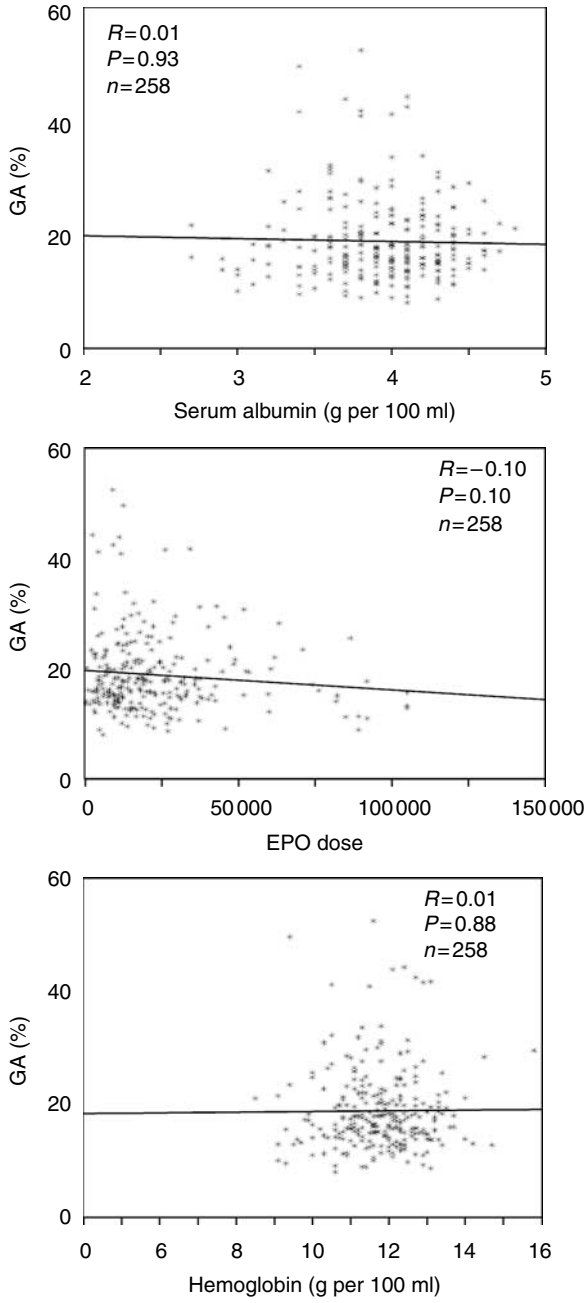

Figure 4 | Impact of hemoglobin concentration, serum albumin and EPO dose on glycated albumin in diabetic ESRD. Relationship between % GA and serum albumin concentration (upper panel); weekly erythropoietin dose (middle panel); and hemoglobin concentration (lower panel) in diabetic subjects on hemodialysis.

dose. As previously noted, % GA is associated with body mass index, a factor for which adjustment can be performed.[16,17] Therefore, % GA may be a more robust indicator of long-term glycemia than HbA_{1c} in hemodialysis patients.

A relative reduction in HbA_{1c} among Japanese patients with type 2 diabetes on hemodialysis was reported by Inaba *et al.*[8] The % GA/HbA_{1c} ratio was 2.93 in those with normal renal function and 3.81 in dialysis patients ($P<0.001$). In the current study, the % GA/HbA_{1c} ratio was 2.07 in American diabetics without severe nephropathy and 2.71 in dialysis patients. Thus, the relationship between % GA/HbA_{1c} ratios in diabetic patients with kidney failure, relative to those without, was nearly identical in Japanese ($3.81/2.93 = 1.30$) and Americans ($2.72/2.07 = 1.31$), revealing that a uniform bias is introduced by lower HbA_{1c} measurements in those on hemodialysis.

The absolute GA value (and % GA) in our study differed from those in the Japanese report due to use of different serum albumin assays.[8] Inaba *et al.* used a new and improved method for albumin measurement.[18] However, the reported difference between the older bromocresol purple methods and the bromocresol green (BCG) in patients undergoing hemodialysis is albumin concentration (BCG in $g\,l^{-1}$) = 5.5 + albumin concentration (bromocresol purple in $g\,l^{-1}$); an approximately 20% lower value compared with the BCG method.[19] On the basis of this, the albumin concentrations in our study should be approximately 20% higher than if the new bromocresol purple method were used. Adjusting our % GA values upward by 20% coincides well with the Japanese results. For example, the % GA/HbA_{1c} was 2.72 and 2.07 in the US participants on hemodialysis and without nephropathy, respectively; translating to 'corrected' values of 3.26 (2.72×1.2) and 2.48 (2.07×1.2) with the new bromocresol purple method. In fact, these estimates approach the Japanese % GA/HbA_{1c} values, 3.81 in hemodialysis patients and 2.93 in nonnephropathy participants. The large number of African Americans in our study, some likely carriers of hemoglobin S, hemoglobin C, and thalassemia (potentially shortening RBC survival during the stress of hemodialysis) may also be associated with slightly different results in our report. Another way to look at these data is through the relative reference ranges, which differ because of the differences in the type of albumin assay. Our US normal % GA range was 11.6% ± 1.6. When multiplied by 20%, the mean % GA becomes 13.9% and coincides well with the reference interval of 12.3–16.9% GA in the report of the Committee on Standardization of Laboratory Testing Related to Diabetes Mellitus of the Japan Diabetes Society: Determination of the Reference Intervals of Hemoglobin A_{1c} (IFCC) and Glycoalbumin in the Japanese Population. According to the recent American College of Pathology surveys, approximately 50% of clinical laboratories in the United States utilize the BCG method for albumin analysis.

As in the present report, the Japanese study detected positive associations between hemoglobin concentration and HbA_{1c}. However, Inaba *et al.*[8] reported that higher serum albumin concentrations were associated with a lower % GA. We did not find such a relationship. Inaba *et al.*[8] also reported that pre- and postdialysis GA results were equivalent, excluding falsely high GA determinations due to uremic toxin accumulation. Finally, it should be mentioned that the dose of erythropoietin in American dialysis patients far exceeded that administered to Japanese dialysis patients. A relationship between higher erythropoietin dose and lower hemoglobin

concentration is well documented in the US dialysis population.

Potential weaknesses in this study include use of random (not necessarily fasting) recent blood glucose measures in participants on dialysis and those without nephropathy, the small number of African American diabetic nonnephropathy participants, and marked ethnic differences among the dialysis patients and those without nephropathy. Fasting blood glucose measurements could not readily be obtained, as patients on the afternoon hemodialysis shift are unable to report fasting, and those on the morning shift are typically away from home for periods exceeding 6 h and are not permitted to eat or drink during hemodialysis. The marked ethnic differences between ESRD participants and non-nephropathy participants reflected the increased risk for nephropathy in African Americans. The relationship between % GA and HbA_{1c} was consistent in the African American nondialysis cohort, as well as in the non-African American (predominantly Caucasian) groups (Table 2). However, ethnic differences in this relationship were observed among subjects on hemodialysis. Finally, these results are predominantly from subjects with type 2 diabetes. Although the results may be generalizable to those with type 1 diabetes mellitus, this will require further study.

HbA_{1c} has long been the assay of choice for determining long-term glycemic control among dialysis patients; therefore, it is crucial that the limitations of this assay be appreciated. Blood glucose tends to decline in diabetic patients with progressive nephropathy due to malnutrition, increased half-life of insulin, and reduced rate of gluconeogenesis. However, the elevated % GA/HbA_{1c} ratio in diabetic dialysis patients, relative to diabetic subjects without nephropathy, strongly suggests that HbA_{1c} was falsely reduced in diabetic subjects on hemodialysis, an effect likely due to shortened RBC survival.

A consistent relationship between the % GA and HbA_{1c} in two independent studies demonstrates that long-term glycemic control in diabetic hemodialysis patients vary based upon the assay used. These reports evaluated more than 1200 diabetic subjects from three ethnic groups. Together, they support % GA as a more accurate test of long-term glycemic control among diabetic hemodialysis patients. It will be important to determine the accuracy of long-term glycemic assays in diabetic subjects with less severe degrees of renal impairment (stages III, IV, and V chronic kidney disease) and whether % GA is an accurate predictor of morbidity and mortality in diabetic subjects on hemodialysis, as in nondialysis populations.[20,21]

MATERIALS AND METHODS

Patients

Subjects with a clinical diagnosis of type 1 or type 2 diabetes mellitus were invited to participate; all received oral hypoglycemic agents, insulin, or both (one dialysis patient had diabetes medications withheld due to recurrent hypoglycemia). Patients with ESRD received hemodialysis treatments and intravenous erythropoietin at Wake Forest University School of Medicine (WFUSM) owned and operated outpatient dialysis units in northwestern North Carolina. Subjects without kidney disease were recruited from WFUSM primary care clinics and denied prior kidney disease, nephrology care, and receipt of erythropoietin or dialysis. Patients on hemodialysis were intentionally over sampled, due to the previously reported disparity between assays of glycemia in this population.

Participants provided demographic and medical information, 10 ml of blood, and access to existing laboratory results. Blood was drawn from the dialyzer circuit in subjects with ESRD, prior to initiation of dialysis or administration of anticoagulants. Blood samples were then divided with 5 ml sent for HbA_{1c} and 5 ml centrifuged with the serum frozen at $-80^{\circ}C$ for future measurement of GA. All subjects provided written informed consent and the study was approved by the Institutional Review Board at the WFUSM.

Information collected from participants included demographic data, height, weight (dry weight in hemodialysis patients), duration of diabetes, smoking status, use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, and random serum glucose concentrations performed in CLIA-certified laboratories. In subjects on dialysis, the minimum and maximum doses of erythropoietin in units over the preceding 3 months were recorded. The dialysis dose (single pool K_t/V; urea reduction ratio), transferrin saturation, ferritin, blood urea nitrogen, serum creatinine, and aspartate aminotransferase concentrations were recorded from the current monthly dialysis lab report.

GA and HbA_{1c} assays

Serum albumin concentrations were measured using the BCG assay, calibrated to the College of American Pathologists. Glycated albumin was measured using the LUCICA GA-L kit (Asahi Kasei Pharma Corporation, Tokyo, Japan) on frozen serum samples (freezer time at $-80^{\circ}C$ ranged from 2 to 68 days). This kit uses an enzymatic method that converts GA to glycated amino acids. The amino acids are then oxidized with the formation of hydrogen peroxide, which is coupled to a dye yielding a purple–blue color. The GA analysis was performed on the automated 'ADVIA 1650' instrument (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). The normal % GA range ($11.6 \pm 1.6\%$) was determined in nondiabetic American volunteers without kidney disease.

HbA_{1c} was analyzed within 24 h of collection (refrigerated if not immediately assayed) on a cation exchange column chromatograph using an automated high-pressure liquid chromatography instrument (Variant II Turbo; Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analyses

The % GA was calculated as (glycated albumin/serum albumin*100). An average of the minimum and maximum erythropoietin doses over the 3 months immediately preceding the study was determined. Descriptive statistics, including means and standard deviations for continuous measures and frequencies and proportions for categorical data, were calculated. Univariate group comparisons were performed via an independent t-test for continuous measures or a χ^2 test for categorical data; hemoglobin group differences were tested by a Wilcoxon two-sample test due to the sparse data in controls ($n = 10$). Pearson's correlation coefficient (R) was used to assess the strength of the relationships between clinical variables. Analysis of variance was used to assess the difference in slope of the lines between study groups, and a

multivariate analysis of variance was used to construct a full model to determine which factors were associated with HbA_{1c} and % GA. Reduced models were created via an iterative backward stepwise procedure, until all remaining variables had a P-value $\leqslant 0.05$.

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