Sickle Cell Trait and Development of Microvascular Complications in Diabetes Mellitus

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Background and objectives: Many African Americans (AA) have both sickle cell trait (SCT) and diabetes mellitus. The objective of this study was to determine whether individuals with diabetes mellitus and SCT have higher rates of microvascular complications relative to those without SCT.

Design, setting, participants, & measurements: This was a retrospective study comparing albuminuria, estimated GFR (eGFR), and microvascular complications in AA with diabetes on the basis of presence of SCT. The study included 821 outpatients who underwent hemoglobin A1c (HbA1c) testing, and presence of SCT was determined using the HbA1c assay. Medical record review and telephone interviews were performed for AA participants.

Results: Data were obtained on 376 AA patients (110 with SCT, 245 with neither SCT nor hemoglobin C trait, and 21 with hemoglobin C trait) and 445 European Americans. The mean eGFR and urinary protein excretion were similar between the three AA subgroups. Analysis revealed that 36.3% of AA nontrait and 22.7% of AA SCT participants had retinopathy, peripheral vascular disease, or end-stage kidney disease ($P = 0.01$). After adjustment for diabetes duration, age, insulin use, and gender, differences in the prevalence of microvascular complications were not observed.

Conclusions: SCT does not increase the risk of microvascular complications in AA with diabetes mellitus.

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Sickle cell trait (SCT) and diabetes mellitus (DM) are common conditions occurring together in more than 1 million individuals worldwide (1–3). Despite the many dually affected individuals, little information exists on whether SCT affects the course and development of complications in patients with DM.

Because patients with SCT and DM are prone to papillary necrosis and hematuria (4), it is possible that pathophysiologic changes induced by SCT could potentiate the microvascular complications of DM. It is unknown whether individuals with SCT face increased risk for developing the microvascular complications associated with DM.

Newer methods for measuring hemoglobin A1c (HbA1c) now quantify hemoglobin S (5–7). Most laboratories do not report this information. However, if obtained, individuals with SCT can readily be identified for research or clinical purposes.

To better understand the interactions between HbA1c and SCT, we studied HbA1c measurements from outpatients at an academic medical center over an 8-month study period. Patients with SCT or hemoglobin C trait were identified based on results of the gas chromatography performed to measure HbA1c. Laboratory values in individuals with and without SCT were contrasted, and surveys were performed for the presence of end-organ damage. We also determined whether individuals with SCT were previously aware of this diagnosis.

Materials and Methods

The study was approved by the Wake Forest University School of Medicine Institutional Review Board.

Laboratory Analysis

HbA1c was analyzed using cation exchange column chromatography on an automated HPLC instrument (Variant II Turbo, Bio-Rad Laboratories, Hercules, CA). This analytic method results in elution of hemoglobin variants and determines the proportion of these variants relative to the total hemoglobin concentration. It has been shown to be a reliable determinant of the hemoglobin S concentration and allows for the determination of hemoglobin S trait and hemoglobin C trait (5–7).

Serum glucose concentrations were determined enzymatically by the hexokinase method on the ADVIA 1650 instrument (Siemens Diagnostic Solutions, Tarrytown, NY). Serum creatinine concentrations were analyzed based on a modified Jaffe reaction (picric acid) using the ADVIA 1650 automated instrument (Siemens Medical Solutions, Tarrytown, NY). Calibration of the creatinine measurement was based on serum standards provided by the manufacturer (Siemens Solution) with equivalence determined by running a College of American Pathologists supported protocol involving samples measured using the National Institute for Standards and Technology isotope dilution mass

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spectroscopy method. The estimated GFR (eGFR) was determined by using the Modification of Diet in Renal Disease equation for standardized serum creatinine concentrations (8).

Urinary albumin and protein excretion measurement was performed on an automated chemistry analyzer (DXC 800, Beckman Instruments, Brea, CA): microalbumin was determined by immunoturbidity and total protein was determined by pyrogallol red.

Data Analysis

Raw data from 1699 analyses of HbA1c from 1590 patients were entered into a Microsoft Excel spreadsheet between May 27, 2008 and August 4, 2008. These data were obtained for all individuals regardless of race. Thereafter, all HbA1c reports with an elevated percentage of hemoglobin S (>20%) were entered from August 5, 2008 through February 27, 2009. Individuals with a hemoglobin S concentration >20% but <50% were considered to have SCT. Hemoglobin C trait was determined in a similar fashion. Individuals homozygous for hemoglobin S, hemoglobin C, or another hemoglobinopathy were excluded. Patient identifiers from these individuals were cross-referenced with the North Carolina Baptist Hospital Clinical Laboratory in Winston-Salem, North Carolina. All individuals >18 years of age were included. For each individual, every simultaneous outpatient measurement of HbA1c, serum glucose, and serum creatinine was captured. In addition, electrolytes, urine protein measurements, and urinalyses between January 2004 and April 2009 were obtained. Blood and urinary measurements were obtained at various times during the day.

Descriptive statistics, including means, medians, SD, and ranges for continuous measures and frequencies and proportions for categorical outcomes, were calculated. Statistical comparisons were performed using ANOVA when testing for differences between study groups; comparisons with a P value <0.05 were considered to be statistically significant.

Study laboratory values were limited to those obtained in the outpatient setting. In some instances, HbA1c was used as a screening test for DM; therefore, in all individuals in whom every HbA1c value was <7%, a chart review was performed to determine if the individual was prescribed hypoglycemic agents or was diagnosed with diabetes. Individuals with a HbA1c value <7% not actively taking insulin or oral hypoglycemic agents and without a diagnosis of DM were excluded from the analysis.

The Modification of Diet in Renal Disease formula was used to determine eGFR (8). To characterize urinary protein excretion, many individuals had a random spot urine protein:creatinine ratio or random spot albumin:creatinine ratio. These values were analyzed in several ways. First, the mean value of all urinary albumin:creatinine ratios for each patient was determined. The median values were then compared between the different groups. Similarly, the mean value for the urinary protein:creatinine ratios for each patient was calculated, and the median value of all urinary protein creatinine values was compared between groups. A comparison was also made between the first urinary protein creatinine values and first urinary albumin:creatinine ratios between groups.

Because some patients had urinary protein:creatinine ratios, and others had urinary albumin:creatinine ratios, we normalized these values by dividing the result by the upper limit of the normal range for each assay. For the urinary albumin:creatinine ratio, the result was normalized by dividing by 30, and for the urinary protein:creatinine ratio, the result was normalized by dividing by 0.2. Again, the median normalized urinary protein:creatinine ratio was compared between groups as well as the initial normalized ratio.

Using the first normalized urinary protein:creatinine ratio, a model was created with the normalized urinary protein:creatinine ratio as the outcome variable and age, gender, initial HbA1c, diabetes duration, and subgroup [African American (AA) with (*1*) SCT without hemoglobin C trait, (*2*) hemoglobin C trait without SCT, and (*3*) without SCT and hemoglobin C trait] as independent variables. In a similar manner, a model was created with eGFR as the outcome variable.

In AA, medical records were reviewed and patients telephoned (up to three calls) to determine DM duration, type of DM, current use of insulin, knowledge of SCT carrier status, history of amputation, and presence of DM retinopathy or gout. Interviewers were a registered nurse and a physician that were experienced interviewers trained by the primary investigator. An interview template was used, and interviewers were blinded to SCT status. Patients were given the opportunity to ask questions if they did not understand a particular question. The educational level of participants was not obtained.

Results

There were 1939 assays performed on 1784 individuals. The data on these individuals were merged with the hospital database for patients with simultaneous HbA1c and glucose measurements performed on an outpatient basis. There were 1256 individuals in this group. Of these, 435 individuals whose HbA1c measurements were all <7% without a clinical diagnosis of DM were removed. There remained 21 AA with hemoglobin C trait, 110 with SCT, 245 with neither hemoglobin S nor hemoglobin C trait (defined as "nontrait"), and 445 European Americans (EA).

Table 1 contains demographic characteristics and initial chemistry values for each group. Each subgroup (SCT, hemoglobin C trait, and EA) was compared with the AA nontrait reference group. Consistent results were obtained when analyses were performed using only the first available determination for each patient, using all chemistry values for all patients, or using only the most recent value. Individuals with SCT were slightly younger than nontrait individuals (54.0 ± 13.9 years *versus* 56.5 ± 13.3 years, $P = 0.025$), which required adjustment in later models. For the AA subgroups, glucose, HbA1c, and eGFR were similar between all three groups. There were several differences between EA and the AA nontrait patients, including differences in gender, HbA1c, serum potassium, and eGFR. There were only 21 individuals in the hemoglobin C trait group, limiting power for comparisons.

There were 528 urine albumin creatinine measurements in 113 individuals, with 21% having one reading, 20% two readings, 16% three readings, 12% four readings, and 31% more than four readings. There were 235 urinary protein:creatinine ratios, of whom 9% had one reading, 18% two readings, 15% three readings, 10% four readings, 11% five readings, and 37% more than 5 readings.

The urinary albumin:creatinine ratios, urinary protein:creatinine ratios, and normalized urinary protein:creatinine ratios were similar between groups, whether the first measurement was compared or the median result of all measurements, except for the hemoglobin C trait group, which had significantly higher urinary albumin:creatinine ratios compared with the AA nontrait group. The first eGFR value was also not different between AA individuals with SCT and nontrait individuals. With adjustment for age, gender, and initial HbA1c in a multivariate model (Table 2), differences were not detected in AA

Table 1. Comparison of characteristics between subgroups (all comparisons were made between individual subgroups and the AA nontrait subgroup)[a]

Subgroup	AA Nontrait	AA SCT	*P* Value AA SCT *versus* AA Nontrait	AA Hemoglobin C Trait	*P* Value AA Hemoglobin C Trait *versus* AA Nontrait	EA	*P* Value for EA *versus* AA Nontrait
N	245	110		21		445	
Age (years), mean ± SD	56.5 ± 13.3	54.0 ± 13.9	0.025	53.8 ± 17.0	0.27	57.6 ± 16.7	0.37
Gender (% male)	31.8	39.1	0.19	38.1	0.63	50.6	0.0001
Glucose							
mmol/L	9.55 ± 5.67	8.60 ± 4.55	0.40	10.1 ± 5.38	0.41	9.10 ± 4.94	0.27
mg/dl	172 ± 102	155 ± 82		181 ± 97		164 ± 89	
HbA1c (%), initial value mean ± SD	8.3 ± 2.2	8.2 ± 1.9	0.63	8.7 ± 2.8	0.29	7.6 ± 1.7	0.005
Serum potassium (mmol/L), mean ± SD	4.20 ± 0.51	4.25 ± 0.48	0.46	4.40 ± 0.61	0.12	4.44 ± 0.53	0.0001
Serum bicarbonate (mmol/L), mean ± SD	27.2 ± 3.1	26.9 ± 2.9	0.38	28.8 ± 2.8	0.042	26.9 ± 3.4	0.30
Blood urea nitrogen, mean ± SD							
mmol/L	6.57 ± 3.8	6.57 ± 5.0	0.99	6.18 ± 3.0	0.70	7.78 ± 4.9	0.0008
mg/dl	18.4 ± 10.6	18.4 ± 14.0		17.3 ± 8.4		21.8 ± 13.7	
Initial eGFR (ml/min/1.73m^2), mean ± SD	69.3 ± 29.7	71.5 ± 28.2	0.49	77.8 ± 24.9	0.17	58.7 ± 25.2	0.0001
Median urinary albumin:creatinine ratio, initial measurement	18.5	24.0	0.23	104	0.006	20.0	0.33
Median of mean of all urinary albumin:creatinine ratio measurements	29.5	45	0.11	113	0.015	35.5	0.11
Proportion of individuals with urinary albumin: creatinine ratio >300	12.8% (n = 156)	16.0% (n = 75)	0.55	38.5% (n = 13)	0.027	12.0% (n = 284)	0.88
Median urinary protein: creatinine ratio, initial measurement	0.40	0.22	0.52	0.29	0.73	0.21	0.52
Median of mean of all urinary protein:creatinine ratio measurements	0.71	0.48	0.98	0.29	0.49	0.47	0.85
Proportion of individuals with a urinary protein: creatinine ratio >0.2	66.2% (n = 71)	62.1% (n = 29)	0.82	60% (n = 5)	0.99	66.9% (n = 130)	0.99
Median normalized urinary protein:creatinine ratio, initial measurement	0.73	0.95	0.3	2.68	0.025	0.77	0.66
Median normalized urinary protein excretion	1.47	1.67	0.45	3.47	0.10	1.52	0.30
Proportion of individuals who ever had hematuria	8/243 (3.3%)	2/105 (1.9%)	0.73	2/20 (10%)	0.17	9/413 (2.2%)	0.45

[a]*P* value is for comparison with reference group of AA without SCT.

Table 2. Multivariate regression model for normalized protein:creatinine ratio

Parameter	Reference Group	Beta Estimate	*P* Value
Intercept		12.37	0.12
Age at measurement	Per 1-year increase	−0.12	0.12
White	AA without SCT or hemoglobin C trait	−2.62	0.33
SCT		−1.05	0.78
Hemoglobin C trait		1.53	0.85
HbA1c (%)	Per 1-unit increase	1.02	0.09
Female	Male	−2.30	0.33

subgroups for initial normalized urinary protein:creatinine ratio.

Similarly, a multivariate model was created with eGFR as the outcome variable (Table 3). After adjustment, there was no difference in eGFR for AA subgroups, although there was a difference between EA and nontrait AA. Table 4 contains the chart review and telephone interview results for AA participants. Nontrait participants were older and more likely to require insulin. They also were more likely to have retinopathy. Thirty-six percent of nontrait AA had peripheral vascular disease, retinopathy, or kidney failure, compared with 23% of SCT patients ($P = 0.01$). However, a logistic regression model adjusting for DM duration, insulin use, gender, and age revealed no effect of nontrait *versus* SCT on the presence of microvascular complications ($P = 0.11$).

Discussion

This investigation provides reassuring information for the many AA patients with SCT and DM. Laboratory studies revealed that the mean percent HbA1c and serum glucose levels were similar between AA participants with and without SCT. Markers of kidney damage, eGFR, and urinary protein:creatinine ratio were also similar between groups.

The presence of microvascular complications was similar between patients with and without SCT after adjustment for DM duration and insulin usage. These results suggest that SCT does not affect the presence or progression of microvascular complications in DM.

Prior studies did not identify relationships between diabetic microvascular complications and SCT, although they included small sample sizes [4 individuals with SCT in a study of retinopathy (9) and 34 with SCT in a study of albuminuria (10)]. One study detected a higher rate of DM complications using a composite score that included retinopathy, foot ulcers, chronic kidney failure, and left ventricular hypertrophy in 16 patients with SCT compared with 36 controls. The study presented here obtained detailed information in 110 AA individuals with SCT.

Several obstacles may have hindered prior studies. First, the proportion of individuals of African descent who have SCT is <10%; therefore, many individuals needed to be tested to identify the small group of affected individuals. Second, hemoglobin electrophoresis is not routinely performed in adult AA, making retrospective studies less likely to identify individuals with SCT. Third, current assays to detect SCT are expensive; therefore, prospective studies would be difficult to perform because many individuals would have to be tested. Ethical issues would also be raised as to whether to inform study participants of their SCT carrier status. By obtaining direct results from the HbA1c assay, we were able to retrospectively identify patients with SCT from a large group of individuals at minimal cost.

Forty percent of patients with SCT were unaware that they were hemoglobin S carriers. Most laboratories using this HbA1c assay do not report to physicians whether individuals had SCT. Although this study indicates that the SCT is not associated with the microvascular complications of DM or interpretation of HbA1c results, SCT may lead to other health problems (hematuria, papillary necrosis, and sudden cardiac death) (4). Whether SCT status should be reported when it is incidentally detected in patients being evaluated for DM control is an ethical question that deserves further study. Most nephrologists, endocrinologists, and hematologists are unaware that the presence of abnormal hemoglobins can be de-

Table 3. Multivariate regression model for eGFR at first measurement

Parameter	Reference Group	Beta Estimate	*P* Value
Intercept		102.36	<0.0001
Age at measurement	Per 1-year increase	−0.76	<0.0001
White	AA without SCT	−7.88	0.0001
SCT		0.56	0.84
Hemoglobin C trait		7.09	0.21
HbA1c (%)	Per 1-unit increase	1.13	0.013
Female	Male	0.91	0.62

Table 4. Results of telephone interview and chart review in AA

	Nontrait	SCT	Hemoglobin C Trait	*P* Value Comparing SCT and Nontrait
N	245	110	21	
Participated in telephone interview (%)	73.1	77.3	61.9	0.40
Duration of diabetes (years)	11.1 ± 7.5	9.41 ± 11.1	11.0 ± 9.8	0.16
Percent requiring insulin	62.0	50.9	61.9	0.05
Percent diagnosed with type 1 diabetes	9.0	7.3	0	0.60
Percent knowing SCT status	17.2%	43.8%	60%	0.0001
Proportion of relatives with sickle cell disease (%)	15.7	31.8	40.0	0.02
Retinopathy (%)	30.6	17.3	33.3	0.008
ESRD (%)	11.8	8.2	0	0.30
Laser surgery (%)	13.9	10.9	9.5	0.43
Blindness in one or both eyes (%)	6.1	3.6	4.8	0.34
Toe amputation (%)	6.94	4.55	4.8	0.39
Foot or leg amputation (%)	4.9	2.7	4.8	0.35
Retinopathy or amputation or dialysis (%)	36.3	22.7	33.3	0.01
Gout (%)	12.2	10.9	14.3	0.72

Table 5. Logistic regression model with presence of microvascular disease (peripheral vascular disease, retinopathy, or kidney failure) as outcome variable

Parameter	Reference Group	Beta Estimate	*P* Value
Intercept		3.18	<0.0001
Duration DM	1 year increase	17.8	<0.0001
Insulin	Not on insulin	12.0	0.0005
Gender	Female	4.2	0.04
SCT	Nontrait	2.62	0.11
Age	1-year increase	2.3	0.13

termined when measuring HbA1c using the routine method that was used in this study.

In many respects, the AA subgroups in this study were ideal for a retrospective case-control study. Many did not know whether or not they had SCT, their physicians did not know, and even if there was knowledge regarding SCT, it was unlikely to affect diagnosis or treatment. Thus, bias was likely minimized in the comparison of AA individuals with and without SCT. However, it is not possible to eliminate all sources of potential bias in a retrospective study, and there may have even been forms of bias of which we are unaware. For example, we obtained our study population by identifying patients who had undergone HbA1c testing. This testing may have been performed more frequently on patients with poorer control or a higher rate of complications. Thus, the patient population may not have been representative of the entire spectrum of diabetic patients, and this potential selective inclusion could have resulted in bias in the results. It is also possible that sicker patients were being treated in our center, thereby causing bias. Finally, it remains possible that SCT patients could have developed more severe diabetes complications leading to premature death, and these individuals were not captured in our sample. Although this could lead to a selected study group, we feel this bias is unlikely because incident and prevalent clinic patients were included in the sample, and adjustment for age in the logistic model failed to alter our observations on the effects of SCT on eGFR or proteinuria.

A shortcoming of the study was the absence of information regarding the use of medications such as angiotensin converting enzyme inhibitors to prevent the development of microvascular complications. These medications could have been taken differentially between the two groups. It is also possible that microvascular complications may be increased in individuals with SCT on no medications, but beneficial medications such as angiotensin converting enzyme inhibitors may decrease microvascular complications in patients with SCT to a similar level as nontrait individuals.

The EA population was significantly different from the AA population, with a higher proportion of men, a lower HbA1c, and a lower eGFR. It is therefore difficult to draw conclusions comparing the AA and EA groups. In contrast, the AA groups with and without SCT were quite comparable and allowed for the comparison of the development of microvascular complications between AA with and without SCT.

In conclusion, this is the largest existing study investigating the presence of microvascular complications in AA individuals

with SCT. It highlights the ability to determine the presence of abnormal hemoglobins during measurement of HbA1c and used this method to study the relationship between SCT and the microvascular complications of diabetes. The presence of SCT did not affect development of diabetic microvascular complications. Finally, many AA were previously unaware that they were hemoglobin S carriers.

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Disclosures

None.

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