Glomerular Involvement in Adults with Sickle Cell Hemoglobinopathies: Prevalence and Clinical Correlates of Progressive Renal Failure

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Patients with sickle cell anemia (SCA) may develop a glomerulopathy with proteinuria and progressive renal insufficiency, leading to ESRD. Albuminuria is a sensitive marker of glomerular damage in this population and precedes the development of renal insufficiency. For determination of the prevalence of glomerular damage in SCA and the clinical correlates of renal insufficiency, 300 adult patients with SCA were studied (hemoglobin SS = 184; and 116 with other sickling hemoglobinopathies: SC, SD, and S-β thalassemia); albumin excretion rates (AER) and renal function (Cockroft-Gault formula) were determined, and clinical and hematologic evaluations were conducted. In hemoglobin SS disease, increased AER (micro- and macroalbuminuria) occurred in 68% of adult patients, and macroalbuminuria occurred in 26%. In other sickling disorders, increased AER occurs in 32% of adults, and macroalbuminuria occurs in 10%. The development of graded albuminuria was age dependent, so at 40 yr, 40% of patients with SS disease had macroalbuminuria. There were no differences in hematologic parameters (hemoglobin levels, white blood cell count, percentage of reticulocytes, platelet counts, or lactate dehydrogenase levels) between patients with normoalbuminuria and those with micro- or macroalbuminuria. By multivariate analysis, albuminuria correlated with age and serum creatinine in SS disease but not with BP or hemoglobin levels. In other sickling disorders, albuminuria tended to be associated with age but not with hemoglobin or BP levels. The diastolic BP was lower in patients with SCA than in African American control subjects, and the development of renal insufficiency, which was present in 21% of adults with SS disease, was not accompanied by significant hypertension. It is concluded that glomerular damage in adults with SCA is very common, and a majority of patients with SS disease are at risk for the development of progressive renal failure. The development of micro- and macroalbuminuria is not related to the degree of anemia, suggesting that sickle cell glomerulopathy is not solely related to hemodynamic adaptations to chronic anemia. In contrast to other glomerulopathies, the development of systemic hypertension is uncommon in SS disease with renal insufficiency.

Sickle cell hemoglobinopathies are a group of genetic disorders that result from a single base-pair DNA mutation in the β globin gene, which leads to the formation of an abnormal hemoglobin tetramer, hemoglobin S ($\alpha_2\beta^S_2$). When the β^S globin gene is inherited in a homozygous pattern (SS disease), it results in a severe disease, with profound anemia and multiple organ involvement, including cerebrovascular events, acute vasoocclusive episodes, retinopathy, acute chest syndrome, and renal damage. Hemoglobin S also may coexist with other mutant β globin chains (β^C or β^D) in a mixed heterozygous state, leading to hemoglobin SC or SD disease. In general, these other sickling hemoglobinopathies have a lesser clinical severity but a similar spectrum of organ involvement. A combined heterozygous condition, S-β thalassemia, occurs when the β^S gene is co-inherited with other mutations in the β^S gene cluster, resulting in β thalassemia (β^O or β^+, depending

the completeness of the lack of β globin synthesis). In S-β thalassemia, the clinical severity of the disease is variable, depending on the relative production of β^S chains.

Hemoglobin SS disease more commonly affects people from African, Mediterranean, or Indian origin. In the United States, approximately 8% of people of African origin are heterozygous for β^S globin, and SS disease occurs in 1 in 400 births. Hemoglobin SC disease is the most common mixed heterozygous form of sickle hemoglobinopathies, occurring in one per 800 births in African Americans. In other parts of the world, the prevalence of sickle hemoglobinopathies is not known as accurately, but it is estimated that in Central and Eastern Africa, the prevalence of the sickle mutation may be as high as 20 to 30%, making sickle cell anemia (SCA) one of the most common inherited diseases in the world (1).

SCA frequently affects the kidney, causing defects in tubulomedullary function (2), but also causes proteinuria, progressive renal insufficiency, and ESRD (3). A glomerulopathy is the cause of the proteinuria and progressive renal insufficiency (4). We previously reported that macroalbuminuria (albumin excretion rates and albumin excretion rates [AER] in excess of 300 mg/g creatinine) results from a glomerular permselectivity

defect, as assessed by dextran sieving analysis, and is associated with a reduction in the two-kidney glomerular ultrafiltration coefficient, even in individuals with preserved GFR (4,5). Therefore, the determination of albuminuria provides a sensitive method to detect glomerular damage in patients with SCA and avoids the pitfalls of estimating glomerular damage from insensitive markers such as serum creatinine.

Previous clinical studies of glomerular damage in SCA have focused on individuals with heavy proteinuria, sometimes in the nephrotic range (6), a relatively uncommon renal presentation of sickle cell disorders, or have studied patients with advanced renal insufficiency (7). In this study, we measured albuminuria as a sensitive marker of glomerular damage to determine the prevalence of glomerular involvement in sickle hemoglobinopathies and to define the course and the clinical correlates of the glomerulopathy. By detecting glomerular dysfunction at an early stage and being able to characterize the broader spectrum of glomerular injury that occurs in sickle hemoglobinopathies, we sought to evaluate the role of anemia and other hematologic alterations in the pathogenesis of sickle cell glomerulopathy and to define other clinical features of the disease.

Materials and Methods
Patient Population

The study was conducted at the Georgia Comprehensive Sickle Cell Center of Emory University. This Center, located at Grady Memorial Hospital, is one of the two Comprehensive Sickle Cell Centers in Georgia (the second center is located in Augusta) and provides care for a large population of patients with SCA (both pediatric and adult). At the time the study was conducted, approximately 1000 active (defined as >1 visit/yr) patients with SCA (550 adults and 450 children) were followed at the center, mainly from the Atlanta metropolitan area. Three hundred adult patients were enrolled in a study of glomerular damage in sickle cell hemoglobinopathies during a 12-mo period. At the time of a routine clinic visit, patients submitted a urine sample for determination of albuminuria. Samples were not collected when patients were acutely ill (including having a fever or needing referral to the urgent care center), had symptoms suggestive of sickle cell pain crisis or urinary tract infection, or had gross hematuria. A total of 184 patients had hemoglobin SS disease, and 116 had other sickling hemoglobinopathies (hemoglobin SC, SD, or S-β thalassemia). Individuals with sickle cell trait (hemoglobin AS) were not studied. In the SS disease group, there were 100 women and 84 men with a median age of 32 yr (range 19 to 71). In the non-SS sickle group, there were 58 women and 58 men, with a median age of 36 yr (range 19 to 76). Clinical, hematologic, and biochemical data were obtained from chart review. For analysis, we averaged the laboratory values of interest that were obtained during the year when the urine specimen was obtained. BP was calculated as the average of the sitting BP measured during each of the last three clinic visits. We excluded from the analysis individuals who were known to be infected with HIV or with a systemic condition that could result in a glomerulopathy not related to SCA (e.g., active hepatitis B or C infections, systemic lupus erythematosus, inflammatory arthropathies), unless glomerular involvement that was related to any of those conditions was excluded by a kidney biopsy.

Urinary albumin was measured using a specific RIA (DPC Laboratories, Los Angeles, CA). Creatinine in urine was measured with a kinetic modification of the Jaffé reaction using a Beckman II creatinine analyzer (Beckman Instruments, Fullerton, CA). AER, expressed as mg/g creatinine, was defined as normoalbuminuria (AER <30 mg/g creatinine), microalbuminuria (AER 30 to 299 mg/g creatinine), or macroalbuminuria (AER $\geq$ 300 mg/g creatinine). Other laboratory values were measured using standard hospital laboratory techniques.

Creatinine clearance was estimated from the serum creatinine, age, weight, and gender, according to the Cockroft-Gault formula (8). We defined renal insufficiency as a creatinine clearance <90 ml/min (9). Control values for BP levels in the African American population were obtained from the published values in the Second National Health and Nutrition Examination Survey (10).

Concomitant Medications, Chronic Transfusions, and Cigarette Smoking

Three patients with SS disease were on antihypertensive medications (one with normoalbuminuria and two with macroalbuminuria, all treated with calcium channel blockers [CCB]). In other sickling disorders, 15 patients were on antihypertensive medications: Five in the normoalbuminuria group (three on CCB and two on diuretics), seven in the microalbuminuria group (four on angiotensin-converting enzyme inhibitors, two on CCB, and one on β blockers), and three in the macroalbuminuria group (one on CCB, one on β blockers, and one on angiotensin-converting enzyme inhibitors).

In regard to other treatments, 10% of patients with SS disease and 2% of patients with other sickling hemoglobinopathies were on hydroxyurea. The percentage of patients who had SS disease and were on hydroxyurea was similar in the albuminuria subgroups: 11, 10, and 8.5% in normo-, micro-, and macroalbuminuria patients, respectively (NS). Only three (2%) of 184 adult patients with SS disease were on a chronic transfusion program, and none of the patients with other sickling hemoglobinopathies was receiving chronic transfusions.

The percentage of patients who smoked cigarettes was 2% (four of 184) in patients with SS disease and 16% (18 of 116) in patients with other sickling hemoglobinopathies. In the latter group, the percentage of smokers was similar in the normo-, micro-, and macroalbuminuria groups: 15, 19, and 17%, respectively (NS).

Statistical Analyses

Values are expressed as mean $\pm$ SEM, unless indicated otherwise. Differences between groups were determined by unpaired t test or ANOVA where appropriate. Proportional differences were analyzed using a contingency table and χ^2 analysis. Association between variables was assessed by correlation coefficient and univariate and multivariate analyses. For the logistic multivariate analysis, the values of AER were log-transformed to approximate a normal distribution. $P < 0.05$ were considered statistically significant.

Results

Clinical and hematologic parameters in patients with SS disease and other sickling hemoglobinopathies are shown in Table 1. Patients with hemoglobin SS have a more severe disease than individuals with other sickling hemoglobinopathies. This is evidenced by a worse anemia (hemoglobin of 8.3 $\pm$ 0.1 in SS disease versus 11.4 $\pm$ 0.2 g/dl in non-SS sickle hemoglobinopathies; $P < 0.05$) and a higher degree of ineffective erythropoiesis (significantly higher lactate dehydrogenase levels and percentage of reticulocytes in SS disease versus non-SS sickle hemoglobinopathies). Other hematologic parameters (white blood cell and platelet counts) also are significantly higher in SS disease than in other sickling disorders. Body weight and body mass index (BMI) also are significantly lower in patients with

Table 1. Clinical parameters[a]

	Age[a] (yr; median [range])	Gender (M/F)	Weight (kg)	Height (cm)	BMI (kg/m²)	Hb (g/dl)	Reticulocytes (%)	Platelets (×10³/μl)	WBC Count (×10³/μl)	LDH (U/L)	SCr (mg/dl)	CrCl (ml/min per 1.73 m²)
SS disease	32 (19 to 71)	84/100	62 ± 1[b]	168 ± 1[b]	22 ± 1[b]	8.3 ± 0.1[b]	13 ± 1[b]	366 ± 10[b]	13 ± 1[b]	511 ± 17[b]	0.86 ± 0.07	132 ± 4[b]
Non-SS sickle hemoglobinopathies	36 (19 to 76)	58/58	75 ± 1	164 ± 2	29 ± 1	11.4 ± 0.2	4 ± 1	274 ± 11	10 ± 1	290 ± 10	0.89 ± 0.03	117 ± 4

[a]BMI, body mass index; CrCl, creatinine clearance; Hb, hemoglobin; LDH, lactate dehydrogenase; SCr, serum creatinine; WBC, white blood cell.
[b]$P < 0.05$ *versus* non-SS sickle hemoglobinopathies.

SS disease *versus* individuals with other sickling diseases, despite a similar age and gender distribution in all sickle hemoglobinopathies. Serum creatinine, on average, is similar in the two groups, but the clearance of creatinine was slightly higher in SS disease than in other sickling disorders (132 ± 4 *versus* 117 ± 4 ml/min per 1.73 m², respectively; $P < 0.05$).

Age and Albuminuria

The prevalence of different degrees of albuminuria in adult patients with SCA is shown in Figure 1. In SS disease, increased AER occurs in 68% of patients: Macroalbuminuria is present in 26%, and microalbuminuria is present in 42%; only 32% of adults with SS disease have normoalbuminuria. In other sickling hemoglobinopathies, the prevalence of increased AER is lower than in SS disease: Abnormal AER occurs in 42% of patients, with macroalbuminuria occurring in 10% and microalbuminuria occurring in 32%. There were no gender differences in the prevalence of albuminuria (data not shown).

Figure 2 shows the prevalence of graded albuminuria according to age. As shown, the development of albuminuria is related in part to age (and therefore, duration of disease). In SS disease, the prevalence of abnormal AER increases from 61% of patients aged 18 to 30 yr to as high as 79% of patients older than 40 yr, so only approximately 20% of patients who have SS disease and are older than 40 yr have normoalbuminuria. More significant, the percentage of individuals with macroalbuminuria doubles between the third and the fifth decades of life: The prevalence of macroalbuminuria is 20% in patients between the ages of 18 and 30 yr but increases to 40% of patients who are older than 40 yr. Similar trends but of a lesser magnitude occur
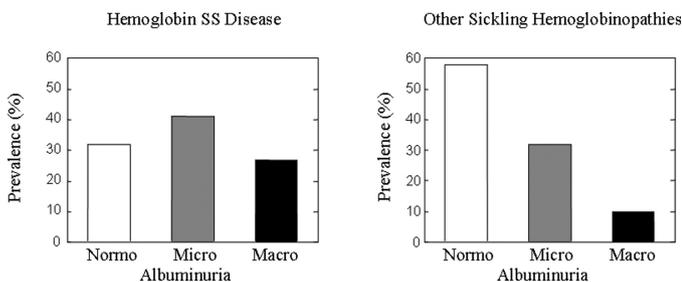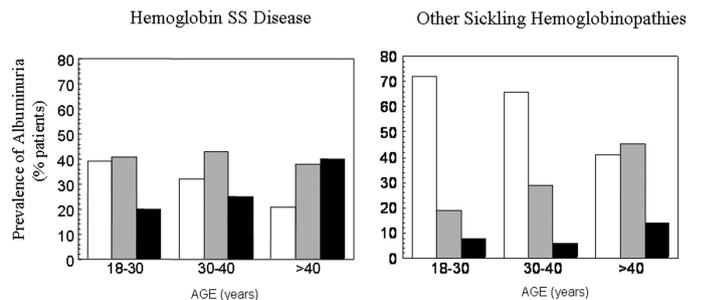
Figure 2. Age and the prevalence of albuminuria in adults with sickle cell hemoglobinopathies. □, normoalbuminuria; ▨, microalbuminuria; ■, macroalbuminuria.

in non-SS sickle hemoglobinopathies: Elevated levels of AER occur in 28% of patients aged 18 to 30 yr but increases to 59% of patients who are older than 40 yr. Macroalbuminuria occurs in 14% of patients with non-SS sickling disorders after the age of 40 yr.

The clinical parameters according to albuminuria levels are shown in Table 2. There were no differences in hemoglobin levels among the groups with different levels of albuminuria for both SS disease and other sickling disorders. For instance, the hemoglobin level in normoalbuminuric patients with SS disease (Table 2) was 8.4 ± 0.2 *versus* 8.1 ± 0.2 g/dl in their counterparts with macroalbuminuria (NS). Similarly, there was no correlation between hemoglobin and AER in SS disease (data not shown). In other sickling disorders (Table 2), the hemoglobin level in patients with normoalbuminuria was 11.5 ± 0.2 *versus* 11.1 ± 0.6 g/dl in patients with macroalbuminuria (NS), and there was no correlation between hemoglobin and AER in non-SS sickle hemoglobinopathies (data not shown). For all sickling diseases, there were no differences in the white blood cell count, platelet count, percentage of reticulocytes, and lactate dehydrogenase levels among patients with normal *versus* abnormal albuminuria.

The relationship between the creatinine clearance and AER is shown in Figure 3. In SS disease, AER tended to increase as creatinine clearance decreased ($r^2 = 0.06$, $P < 0.05$), but there was a large variability, and a significant number of patients had increased AER despite a preserved creatinine clearance. Only one patient with a creatinine clearance <50 ml/min per 1.73 m² had normal AER. In contrast, in other sickling disorders, the

Figure 1. Prevalence of albuminuria in adults with sickle cell hemoglobinopathies. □, normoalbuminuria; ▨, microalbuminuria; ■, macroalbuminuria.

Table 2. Clinical and hematologic parameters in patients with SS disease and other non-SS sickling hemoglobinopathies according to albuminuria[a]

	Age (yr; median)	BMI (kg/m²)	SBP (mmHg)	DBP (mmHg)	SCr (mg/dl)	Hb (g/L)	MCV (fl)	CrCl (ml/min per 1.73 m²)	Reticulocytes (%)	LDH (U/L)
SS disease										
normo	29	22.6 ± 0.7	121 ± 2	62 ± 1	0.7 ± 0.1	8.4 ± 0.2	93 ± 1	132 ± 5	11 ± 1	483 ± 26
micro	32	20.9 ± 0.7[b]	124 ± 2	63 ± 1	0.7 ± 0.1	8.2 ± 0.2	94 ± 2	129 ± 6	14 ± 1	550 ± 36
macro	36	22.8 ± 0.9	127 ± 2[b,c]	69 ± 1[b,c]	1.2 ± 0.2[b,c]	8.1 ± 0.2	95 ± 2	116 ± 7	13 ± 1	471 ± 23
Other sickling diseases										
normo	33	29.4 ± 0.9	127 ± 2	73 ± 2	0.8 ± 0.1	11.5 ± 0.2	83 ± 1	127 ± 5	4 ± 1	284 ± 13
micro	41	27.2 ± 1.1	131 ± 3	75 ± 2	0.9 ± 0.1	11.4 ± 0.3	86 ± 2	112 ± 5	4 ± 1	286 ± 18
macro	43[b]	30.8 ± 1.2	147 ± 6[b,c]	86 ± 3[b,c]	1.2 ± 0.2[b,c]	11.1 ± 0.6	87 ± 2	107 ± 15	5 ± 1	330 ± 46

[a]DBP, diastolic BP; SBP, systolic BP; MCV, mean corpuscular volume.
[b]$P < 0.05$ *versus* normoalbuminuria.
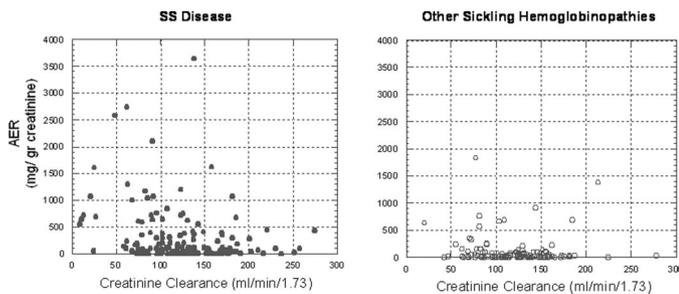[c]$P < 0.05$ *versus* microalbuminuria.

Figure 3. Correlation between creatinine clearance and albumin excretion rate (AER) in SS disease (left; $r^2 = 0.06$, $P < 0.05$) and in other sickling hemoglobinopathies (right; NS).

Figure 4. Relationship between hemoglobin levels and creatinine clearance in SS disease (left; $R^2 = 0.09$, $P < 0.05$) and in other sickling hemoglobinopathies (right; NS). Of note, there is no relationship in SS disease between hemoglobin and creatinine clearance when only patients with normal renal function (creatinine clearance > 90 ml/min) are considered.

AER did not correlate with the creatinine clearance, and fewer patients had advanced renal insufficiency (see next section).

Renal Insufficiency in SCA

Renal insufficiency, defined as a creatinine clearance <90 ml/min, was present in a similar percentage of individuals with SS disease (38 [21%] of 184) *versus* other sickling disorders (31 [27%] of 116; NS). However, the percentage of patients with renal insufficiency and advanced kidney failure (chronic kidney disease stage 3 or higher) was higher in SS disease (11 [29%] of 38) *versus* other sickling disorders (two [6%] of 31; $P = 0.06$). In SS disease, there was a weak correlation between hemoglobin levels and creatinine clearance ($r^2 = 0.09$, $P < 0.05$; Figure 4); however, this correlation was no longer present when the analysis was restricted only to patients with preserved renal function (creatinine clearance > 90 ml/min). In other sickling disorders, there was no correlation between hemoglobin and creatinine clearance (Figure 4).

Anemia and Renal Insufficiency

In patients with SCA and renal insufficiency, the average hemoglobin level was 1.1 to 1.2 g/dl lower than the hemoglobin level of those with preserved renal function ($P < 0.05$ for both SS and other sickling diseases; Table 3). In patients with SS
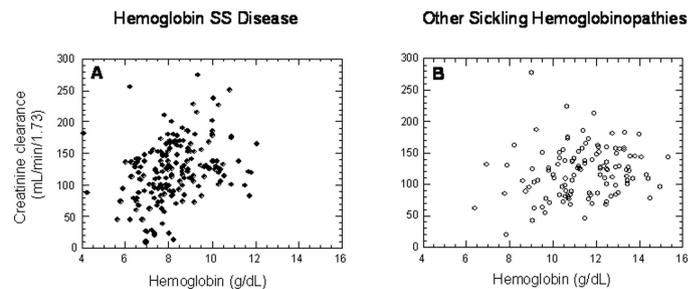
disease and renal insufficiency, there was a correlation between the percentage of reticulocytes and the creatinine clearance ($r = 0.60$, $P < 0.05$). The percentage of reticulocytes in patients with SS disease and more advanced renal insufficiency (creatinine clearance < 50 ml/min) was 50% lower than in patients with creatinine clearance 50 to 90 ml/min (6 ± 3 *versus* 12 ± 4%, respectively; $P < 0.05$), despite similar hemoglobin levels (7.1 ± 0.2 *versus* 7.4 ± 0.5 g/dl, respectively; NS; data not shown).

BP in SCA

Systemic BP levels in patients with sickle hemoglobinopathies are shown in Figure 4. In patients with SS disease, the diastolic BP (DBP) levels were 15 to 20 mmHg lower than those reported in a general population of African Americans in the Second National Health and Nutrition Examination Survey study, but the systolic BP (SBP) levels were similar in patients who had SS disease and were aged 18 to 45 yr *versus* nonanemic African American control subjects. After age 45, however, the SBP levels became significantly lower in patients with SS disease than in African American control subjects (Figure 5). In patients with non-SS sickle hemoglobinopathies, who have a

Table 3. Renal insufficiency in sickle cell anemia[a]

	Age (yr; median [range])	AER (mg/g creatinine)	CrCl (ml/min)	BMI (kg/m²)	SBP (mmHg)	DBP (mmHg)	Weight (kg)	Hb (g/dl)	Reticulocytes (%)	LDH (U/L)
SS disease										
CrCl > 90	30 (19 to 59)	220 ± 36	137 ± 3	22.1 ± 0.4	124 ± 1	64 ± 1	64 ± 1	8.5 ± 0.1	13 ± 1	509 ± 19
CrCl < 90	37[b] (22 to 65)	626 ± 32[b]	56 ± 8[b]	21.9 ± 0.7	125 ± 1	67 ± 1	55 ± 1[b]	7.3 ± 0.3[b]	12 ± 4	521 ± 8
Other sickling hemoglobinopathies										
CrCl > 90	35 (19 to 61)	97 ± 22	130 ± 3	30.1 ± 0.8	129 ± 1	75 ± 1	77 ± 1	11.6 ± 0.2	4 ± 1	291 ± 12
CrCl < 90	54[c] (22 to 68)	222 ± 106[c]	64 ± 4[c]	26.7 ± 1.3[c]	136 ± 6[c]	76 ± 4	67 ± 3[c]	10.5 ± 0.4[c]	4 ± 1	276 ± 22

[a]AER, albumin excretion rate.
[b]$P < 0.05$ *versus* SS disease with CrCl >90 ml/min.
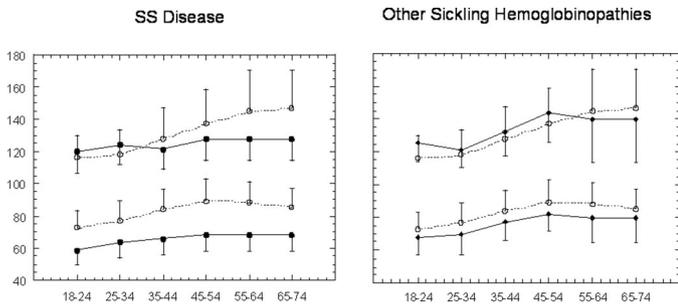[c]$P < 0.05$ *versus* other sickling hemoglobinopathies with CrCl >90 ml/min.

Figure 5. BP levels (mean ± SD) in sickle cell disease (left) and in other sickling hemoglobinopathies (right) compared with African American control subjects (O, Second National Health and Nutrition Examination Survey).

lesser degree of anemia than patients with SS disease, the SBP levels were similar to those of the African American population for all age groups. However, the DBP levels tended to be lower than those in African Americans control subjects, with differences of 6 to 8 mmHg for all ages.

We compared systemic BP levels according to the degree of albuminuria in SCA. The results are shown in Table 2 and Figure 6. In non-SS sickling disorders, macroalbuminuria was accompanied by an increase in the SBP and DBP levels of 20 and 13 mmHg, respectively, *versus* their normoalbuminuric counterparts (Table 2, Figure 6). In contrast, patients with SS

disease and macroalbuminuria had a much smaller increase in BP than normoalbuminuric patients with SS disease: The average increase in systolic and DBP was of only 6 and 7 mmHg, respectively (Table 2), and, as shown in Figure 5, this increase occurred only in patients who were older than 40 yr. In patients who had SS disease and were younger than 40 yr, the development of macroalbuminuria was not accompanied by an increase in BP levels.

The development of renal insufficiency also is associated with a resistance to the development of hypertension in SS disease. As shown in Figure 7 and Table 3, the BP levels are similar in patients who have SS disease with and without renal insufficiency. Only individuals with advanced renal insufficiency (creatinine clearance < 30 ml/min) have systemic BP levels higher than those of other individuals with SS disease (their mean arterial pressure was 93 *versus* 84 mmHg in patients with SS disease and no renal failure; $P < 0.05$). In other sickling disorders, by contrast, renal insufficiency is associated with higher systemic BP levels.

Clinical Correlates of Albuminuria and Renal Insufficiency

To determine clinical or biochemical markers associated with development of albuminuria and renal insufficiency, we performed a multiple linear regression analysis, using albuminuria or creatinine clearance as the dependent variables. When albuminuria was considered, only age ($P = 0.048$) and, to a lesser degree, creatinine clearance ($P = 0.072$) reached or approached statistical significance in SS disease (Table 4); in other sickle
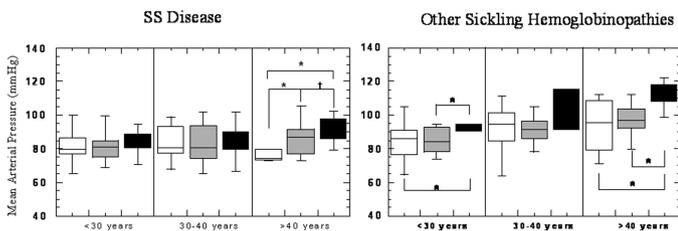

Figure 6. Box plot of mean arterial BP levels according to the degree of albuminuria (□, normoalbuminuria; ▨, microalbuminuria; ■, macroalbuminuria) and age in SS disease (left) and in other sickling hemoglobinopathies (right). *$P < 0.05$ *versus* macroalbuminuria; †$P < 0.05$ *versus* control subjects.
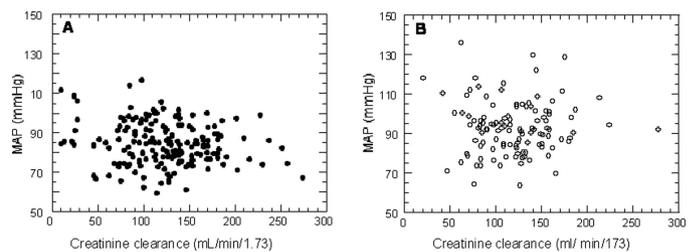

Figure 7. Lack of relationship between creatinine clearance and mean arterial pressure (MAP) in SS disease (A) and in other sickling disorders (B).

Table 4. Multiple linear regression analysis: Albuminuria and CrCl

	Albuminuria (P)		CrCl (P)	
	SS Disease	Other Sickling Disorders	SS Disease	Other Sickling Disorders
BMI	0.732	0.939	0.0568	0.001
Age	0.048	0.065	<0.001	<0.001
AER	—	—	0.378	0.485
CrCl	0.072	0.108	—	—
SBP	0.843	0.026	0.949	0.341
DBP	0.779	0.496	0.182	0.545
Hb	0.923	0.866	0.112	0.957
Reticulocytes	0.205	0.982	0.214	0.613
MCV	0.679	0.453	0.862	0.942
WBC count	0.646	0.859	0.799	0.787
Platelet count	0.302	0.715	0.833	0.222
LDH	0.469	0.767	0.808	0.217

disorders, only SBP ($P = 0.026$) and age ($P = 0.065$) were associated with albuminuria. When we analyzed renal insufficiency as a dependent variable, both age and BMI were associated with a reduction in creatinine clearance in both SS disease and other sickling hemoglobinopathies (Table 4).

Discussion

Previous studies of renal involvement in SCA have emphasized defects in tubulomedullary function or have focused on patients with nephrotic-range proteinuria, an uncommon presentation in SCA. We previously showed that renal insufficiency in SCA results from a glomerulopathy, which can be detected by the presence of albumin and other large molecular weight proteins in urine. In this study, we sought to determine the prevalence of sickle cell glomerulopathy in the adult SCA population and to define the clinical and hematologic correlates of the disease and its progression. We found that glomerular involvement is extremely common in sickle cell hemoglobinopathies: Increased AER occurs in approximately 70% of adults with hemoglobin SS disease and in approximately 40% of adults with other sickling disorders. Moreover, the prevalence of graded albuminuria is age dependent, so after the age of 40 yr, 79% of patients with SS disease have abnormal levels of albuminuria and 40% have macroalbuminuria. This indicates that sickle cell glomerulopathy occurs in a majority of older adults with SS disease, and its prevalence is much higher than previously reported on the basis of a positive urinary dipstick for protein (11).

Can the results from our study be extrapolated to the sickle cell disease population as a whole? As described in Materials and Methods, the Georgia Sickle Cell Center is the only comprehensive sickle cell center in the Atlanta metropolitan area, providing care for a large number of patients with sickle cell disease. To analyze potential biases in patient enrollment, we reviewed the charts of all clinic visits during the period of 1 mo during the year when our study was conducted. Of the patients who met enrollment criteria, urine samples for albuminuria determination were collected in >80% of them. We also com-

pared the prevalence of dipstick proteinuria in the urinalysis that was conducted as part of an annual visit in a separate sample of 342 adult patients who were seen 1 to 2 yr before our study was conducted. We found trace proteinuria in 31 (9%) patients and ≥1+ proteinuria in 67 (19%) of them. These values are comparable to the prevalence of macroalbuminuria (dipstick positivity) in our study cohort and similar to the prevalence of dipstick proteinuria by Falk *et al.* (11) in their series from Chapel Hill, NC. Therefore, we believe that our albuminuria prevalence data are a reflection of the true prevalence of albuminuria in the general sickle cell population.

The pathogenesis of glomerular damage in SCA is not well understood. Children with SCA have renal hemodynamic alterations (renal hyperperfusion and glomerular hyperfiltration) that probably result from renal vasodilation associated with chronic anemia. In some patients, these changes are followed by the development of glomerular proteinuria and progressive renal insufficiency. Histologically, patients with SCA may develop glomerular hypertrophy and focal segmental glomerulosclerosis, features that are suggestive of hemodynamically mediated injury (11). Moreover, short-term administration of enalapril reduced proteinuria without lowering BP or GFR, suggesting that angiotensin II mediates sickle cell glomerulopathy. The cause(s) of the hemodynamic injury to the glomerulus in SCA is unclear. Anemia *per se* could cause glomerular damage by increasing blood flow. In support of this, the prevalence of albuminuria in SS disease (with lower hemoglobin than in other sickling disorders) is higher than in other sickling hemoglobinopathies. Alternatively, other factors that are related to the rheology or stickiness of the sickle erythrocyte could cause glomerular damage, independent of or in conjunction with the hemodynamic changes that are associated with anemia. In support of the latter mechanism, we found that patients with α-thalassemia and SCA have a lower prevalence of albuminuria than their nonthalassemic SS counterparts, despite similar levels of anemia in both groups (12).

In our study, we did not find a correlation between AER and hemoglobin levels. At first, this could be interpreted as an

indication that anemia is not implicated in the pathogenesis of the disease. However, this conclusion cannot be inferred from our study. First, our study is cross-sectional, and hematologic values in adults may be different from those of children. Longitudinal studies in children suggest that the level of hemoglobin tends to remain stable over time for a particular patient (13), but this has not been studied in adults. Second, not all children who are born with SCA reach adulthood; therefore, a study in adults has an intrinsic bias related to early mortality in, presumably, sicker patients. Third, other variables (*e.g.*, genetic polymorphisms, α-thalassemia) could have an impact on the development of the disease and affect the analysis of the results. It also is not known whether there are hemodynamic differences between patients with graded albuminuria; therefore, the role of glomerular hemodynamics in the pathogenesis of sickle cell glomerulopathy still remains to be fully determined. However, when taken together, our findings support that other factors besides the degree of anemia are implicated in the development of sickle cell glomerulopathy.

What is the significance of abnormal albuminuria in SCA? Macroalbuminuria in SCA is the clinical manifestation of an underlying glomerulopathy; this has been confirmed by both physiologic (4) and pathologic studies (11). Using functional techniques, we studied 24 macroalbuminuric patients with SCA and preserved or depressed GFR (inulin clearance) (4). We found that the glomerular ultrafiltration coefficient was severely depressed in patients with renal insufficiency and accounted for the low GFR. In patients with macroalbuminuria but preserved GFR, the glomerular ultrafiltration coefficient also was reduced *versus* normoalbuminuric sickle cell control subjects, indicating that macroalbuminuria, irrespective of the level of GFR, reflects an underlying glomerular pathology. This has been confirmed by the histologic findings in a group of 10 macroalbuminuric patients, reported by Falk *et al.* (11), who had underwent a kidney biopsy. In all instances, features of an underlying glomerulopathy were found. This also is supported by our findings in a group of 25 patients who had SCA and underwent a kidney biopsy because of proteinuria (macroalbuminuria). Again, in all instances, an underlying glomerular pathology was found (unpublished observations).

The clinical significance of microalbuminuria is unclear because no studies of glomerular function or kidney pathology have been performed in this group of individuals. Moreover, the clinical implications of microalbuminuria cannot be fully determined from this cross-sectional study. Therefore, it is not known whether patients with microalbuminuria will progress to macroalbuminuria. However, indirect evidence in children suggests that microalbuminuria could precede the development of macroalbuminuria in adults (14,15). In children, the development of microalbuminuria follows an age-dependent manner. For instance, Dhranidharka *et al.* (14) reported that microalbuminuria was not present in children who were younger than 7 yr but reached 43% in the second decade of life. In a similar study by Wigfall *et al.* (15), the authors found an age-dependent occurrence of dipstick proteinuria: Proteinuria was not present in children who were 0 to 6 yr of age, but it occurred in 7% of children who were aged 7 to 10 yr and in 10%

of children who were aged 13 to 17 yr. The increasing prevalence of albuminuria in children is consistent with our findings in adults. Therefore, it is tempting to speculate from our cross-sectional study and the available literature that sickle cell glomerulopathy could evolve in five clinical stages: (*1*) A normoalbuminuric stage of variable duration, followed by a stage of (*2*) microalbuminuria; this could lead to (*3*) macroalbuminuria but with preserved GFR, and to (*4*) macroalbuminuria and progressive renal insufficiency and (*5*) ESRD. However, evidence of progression from micro- to macroalbuminuria is lacking, and such classification remains a hypothesis. It also is worth noting that there is a large variability in the age when clinical glomerulopathy manifests. This could be related, in part, to genetic factors (12).

Our study in adults also indicates that the prevalence of renal insufficiency in sickle cell disorders is much higher than previously reported (4 to 7%, based on elevated serum creatinine values [7,11]). Using the Cockroft-Gault estimation of the creatinine clearance, we found that renal insufficiency, defined as creatinine clearance <90 ml/min according to Kidney Disease Outcomes Quality Initiative guidelines (8,9), occurs in 21% of adult patients with sickle cell disorders. The true prevalence of renal insufficiency in SCA is difficult to estimate from available methods, but it probably is higher. The Cockroft-Gault equation was derived from estimations of creatinine production on the basis of gender, age, and weight (8). It is not known whether it overestimates creatinine production in patients who have SCA, who have a low muscle mass and a low BMI. Moreover, the Cockroft-Gault formula does not take into account tubular secretion of creatinine, which averages 40% in patients with SCA even when renal function is preserved (4). Therefore, the Cockroft-Gault formula could systematically overestimate true GFR. Unfortunately, other methods, such as the Modification of Diet in Renal Disease formula (16), have not been validated in SCA. To our knowledge, no studies have compared derived with measured GFR in patients with SCA.

We found that systemic BP levels in patients with SCA are lower than that of African American control subjects, as reported previously (17,18). The novel finding of our study is that systemic BP does not increase in a majority of patients with SS when they develop proteinuria or renal insufficiency. In contrast, in non-SS sickling disorders, BP levels increase when albuminuria develops. The mechanism(s) that mediates these hemodynamic changes and the relative resistance to hypertension in patients with SS and renal insufficiency are not known. In transgenic mice that express the human sickle hemoglobin gene, BP is lower than that of their normal littermates, as we found in humans, and there is indication of activation of the nitric oxide (NO) system, as assessed by higher plasma and urinary levels of the NO metabolites (19), increased endothelial NO synthase expression in the systemic vasculature, and blunted arteriolar response to NO-mediated vasodilators (20). This activation of the NO system could be implicated in the resistance to hypertension. In humans with SCA, the urinary excretion of NO metabolites is higher than in nonanemic control subjects (21), and the peripheral vessels show a decreased response to blockade of the NO system, suggestive of chronic

NO activation (22). However, the role of the NO system in patients with SCA and the possible mechanisms of resistance to hypertension remain to be determined.

Conclusion

The findings of this study have important clinical implications. First, the prevalence of glomerular damage in SCA is much higher than previously thought, and a majority of patients with SS disease are at risk for the development of progressive renal insufficiency and late renal failure, especially because the life expectancy in patients with SS disease has improved with better care. Second, in contrast to most glomerular diseases, the glomerulopathy in SS disease is not accompanied by the development of significant systemic hypertension. Therefore, treatments that aim just to reduce systemic BP levels in nonhypertensive individuals are unlikely to be beneficial or tolerated. Other treatment strategies, possibly directed at reducing albuminuria (*e.g.*, angiotensin II blockade), treating the underlying disease, or targeting other potential mechanisms of glomerular damage, will need to be studied. Third, our study suggests that the hemodynamic changes that are associated with chronic anemia *per se* are not solely responsible for the development of sickle glomerulopathy and indicates that other mechanisms are involved in the pathogenesis of the glomerular damage in this population.

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