Early detection and the course of glomerular injury in patients with sickle cell anemia

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Early detection and the course of glomerular injury in patients with sickle cell anemia. We performed a cross sectional analysis of glomerular function in 34 adult patients with sickle cell anemia (SSA). Patients were divided according to GFR and albumin excretion rate (AER): SSA controls (normal GFR and AER, N = 10), albuminuria (increased AER, but normal GFR, N = 7) and chronic renal failure (CRF, low GFR, N = 17). GFR did not correlate with age (that is, duration of disease), but was inversely related to AER and IgG excretion rates (r = -0.61 and -0.69, respectively, P < 0.001) and directly related to the hematocrit (r = 0.55, P < 0.001). Renal plasma flow was disproportionately higher than GFR, so that filtration fraction was low in all groups. Albuminuria was accompanied, even in patients with normal GFR, by a reduction in ultrafiltration coefficient (16 ± 3 in albuminuria vs. 25 ± 3 in controls, P < 0.05). A more severe loss of ultrafiltration coefficient and glomerular permselectivity occurred in CRF. We conclude that renal failure in SSA occurs because of glomerular injury with loss of ultrafiltration coefficient and glomerular permselectivity. The earliest clinically detectable abnormality is an increase in albumin and IgG excretion. When albuminuria is present, the ultrafiltration coefficient is already diminished even if GFR is preserved. Detection of albuminuria can identify established glomerular injury in SSA.

Sickle cell anemia (SSA) has been linked to a variety of abnormalities in renal tubular and medullary function, including defects in urine concentration and acidification [1, 2]. These abnormalities are thought to be caused by renal interstitial and medullary ischemia, which in turn results from erythrocyte sickling and obliteration of the vasa recta [3]. In a subset of patients with SSA, some investigators have emphasized the development of proteinuria and chronic renal insufficiency (CRF), features suggestive of glomerular involvement [4–7]. Once significant proteinuria or CRF are present, the prognosis for SSA patients is poor: 50% will reach end-stage renal disease (ESRD) in less than two years [6]. In addition, CRF is associated with more episodes of sickle cell crises, the acute chest syndrome and strokes [8], and is a predictor of early death [9]. Despite the widespread availability of dialysis, the median survival of SSA patients after the diagnosis of renal failure is only four years [8].

The course of glomerular injury and renal insufficiency in SSA has not been characterized. Renal function in children with SSA is characterized by glomerular hyperfiltration and hyperperfusion [10], but as early as the second decade of life GFR often declines, despite the persistence of high renal blood flow rates [11]. In patients with mild renal insufficiency and minimal proteinuria, a renal interstitial process has been proposed as the cause of these abnormalities [12]. More commonly, however, there is a pattern of proteinuria and progressive renal insufficiency, suggesting glomerular involvement [5–7]. In patients with nephrotic-range proteinuria, global and segmental glomerulosclerosis [7, 13] or mesangial proliferation [14–17] has been described. The aim of our study was to determine the course of progressive renal insufficiency in patients with SSA and, using physiological techniques, to identify possible markers of early glomerular injury in this disease.

Methods

Patients

All adult patients with SSA followed at the Georgia Comprehensive Sickle Cell Center were eligible to participate in a study of glomerular function that was approved by the Human Subjects Committee of Emory University. Both patients with and without known renal disease were contacted. Thirty-six patients (13 males) consented to the study. At the time of the examination, no patient had macroscopic hematuria and all were pain-free. Patients who subsequently were found to have proteinuria or renal insufficiency underwent renal ultrasonographic evaluation and had negative serologies for hepatitis B, C and HIV, and normal ANA and complement levels. These tests were negative except for two patients, one with papillary necrosis by ultrasonography and another with recurrent pyelonephritis and they were excluded. Of the 34 patients studied, 23 were homozygous for hemoglobin S and 11 had hemoglobin SC. Their median age was 33 years (range 18 to 65).

Patients were divided in three groups according to GFR and protein excretion rates: SSA controls (normal GFR and normal albumin excretion rate); albuminuria (increased albumin excretion rate, but normal GFR for age); and chronic renal failure (depressed GFR for age). One hundred and two healthy adults between the ages of 18 to 80, we studied earlier, provided control values for GFR and protein excretion rates [18].

Glomerular function studies

Diuresis was established with an oral water load (15 ml/kg) and maintained by drinking a volume equivalent to that of voided urine. Priming doses of insulin (50 mg/kg) and para-aminobiphenyl acid (PAH, 12 mg/kg), followed by an infusion to achieve constant plasma levels of 20 and 1.5 mg/dl, respectively, were administered.
Sixty minutes later, blood pressure was measured and blood was obtained to determine plasma oncotic pressure and serum protein concentrations. Four spontaneously voided, timed urine collections were obtained, with blood samples at the beginning and end of each period. GFR and RPF were calculated as the average of the four urinary inulin and PAH clearances, respectively. RPF was calculated by dividing the clearance of PAH by an extraction ratio. In SSA patients with normal GFR, we used the reported extraction of PAH (E_{PAH}) of 0.85 [19]. In SSA patients with CRF, no data on PAH extraction are available. Based on measured E_{PAH} in patients with other glomerular diseases and renal insufficiency [20], we used a value of 0.70. Filtration fraction (FF) was calculated as the ratio between GFR and RPF.

Inulin and PAH were determined by the resorcinol and Marshall's methods, as described [18]. Plasma oncotic pressure was measured by membrane osmometry (Wescor Oncometer, Logan, Utah, USA) and was assumed to be equivalent to afferent arteriolar oncotic pressure (\(\pi_A\)). Efferent oncotic pressure (\(\pi_E\)) was calculated as:

\[
\pi_E = \pi_A (1 - FF)
\]

glomerular capillary oncotic pressure (\(\pi_{GC}\)) as the arithmetic mean of \(\pi_A\) and \(\pi_E\) [21]. Creatinine was measured using a Beckman II Creatinine Analyzer and serum albumin and IgG concentrations were measured by a turbidimetric technique using specific antibodies (Turbitime System, Behring Diagnostics, Somerville, NJ, USA). Urinary albumin and IgG were measured by a radioimmunoassay (Diagnostic Products Co., Los Angeles, CA, USA) and a sensitive ELISA technique [22], respectively.

The rate of glomerular filtration is governed by the relationship between ultrafiltration coefficient and net ultrafiltration pressure according to:

\[
GFR = K_f \times P_{UF} = K_f \times [\Delta P - \Delta\pi]
\]  

[Eq 1]

where \(K_f\) is the two-kidney ultrafiltration coefficient and \(P_{UF}\) is the net ultrafiltration pressure (the difference between the transglomerular capillary hydrostatic pressure (\(\Delta P\)) and mean glomerular oncotic pressure, \(\Delta\pi\)). A mathematical model for the filtration of water was used to compute \(K_f\) from values of GFR, RPF, \(\pi_A\) and \(\Delta P\) [23]. Three of these variables (GFR, RPF and \(\pi_A\)) were measured; the fourth, \(\Delta P\), cannot be measured in humans. Based on animal studies [24] and indirect evidence in humans [25], a physiologic range for \(\Delta P\) in humans has been estimated between 35 to 40 mm Hg. We computed \(K_f\) at a \(\Delta P\) of 35 mm Hg in all patients to provide an upper boundary for \(K_f\) (maximum \(K_f\)). Since CRF in animal models is almost invariably associated with intraglomerular hypertension [26–28], we also computed \(K_f\) in the albuminuria and CRF groups at a \(\Delta P\) of 40 mm Hg to evaluate the effect of intraglomerular hypertension on \(K_f\).

Statistical analysis

Results are expressed, unless otherwise indicated, as mean ± SEM. Rates of protein excretion and fractional protein clearances were log-transformed to approximate a normal distribution, prior to statistical analysis. Comparisons among the groups were performed with an ANOVA, using Scheffe's correction. Values are considered significant when \(P < 0.05\).

Results

Serum creatinine values < 1.3 mg/dl (115 \(\mu\)mol/liter) corresponded to GFR values ranging from 30 to 160 ml/min/1.73 m² (Fig. 1A). Serum creatinine was abnormally increased only at GFRs < 30 ml/min/1.73 m². Because of tubular secretion, creatinine clearance overestimated simultaneously measured GFR (Table 1), even in those patients with normal function, emphasizing the pitfalls of estimating GFR from serum creatinine [29]. When using the Cockcroft and Gault equation to calculate creatinine clearance from serum creatinine [30], the calculated creatinine clearance was found to be correlated with the measured creatinine clearance (\(r = 0.899, P < 0.001\)), but it also overestimated true GFR, on average by 49%. As depicted in Figure 1B, there was no relationship between GFR and age, and therefore, duration of anemia. GFR was less affected in patients with hemoglobin SC (Fig. 1B), but GFR differences could not be accounted for solely by hemoglobin type. GFR was directly correlated with the hematocrit (\(r = 0.56, P < 0.001\), Fig. 1C).

In the albuminuria group, the average albumin excretion rate was increased by almost 40-fold as compared to SSA controls (Table 2). Albuminuria was accompanied by increased IgG excretion (\(P < 0.001\)). More severe loss of glomerular permselectivity

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**Fig. 1.** A. The relationship between serum creatinine and GFR is shown, with a normal level of 1.3 mg/dl (115 \(\mu\)mol/liter) indicated by a dashed line. Symbols are: (●) Hb SS; (○) Hb SC. B. GFR values are plotted as function of age (that is, duration of disease). C. Individual GFR values are related to hematocrit (\(P < 0.001\)).
was present in CRF patients. Serum protein concentrations tended to fall with increasing proteinuria, but were not statistically different among the groups.

Glomerular hemodynamics are summarized in Table 3. Mean blood pressure was only 82 mm Hg in SSA patients with no renal abnormalities, and somewhat higher in patients with glomerular dysfunction (albuminuria and CRF groups). With progressive proteinuria, intraglomerular capillary oncotic pressure (P_{OC}), the force opposing glomerular filtration, tended to decrease in parallel with plasma oncotic pressure. Renal plasma flow was increased out of proportion to the GFR, indicated by a low filtration fraction in controls. In the CRF group, the loss of GFR was more pronounced than the reduction in renal plasma flow, thus filtration fraction tended to be lower than in controls (Table 3). In the albuminuria group, despite a normal GFR, there was a striking reduction in the computed ultrafiltration coefficient, K_{f}, compared to SSA controls (16 ± 3 vs. 25 ± 3, respectively, P < 0.05). The loss of ultrafiltration capacity, reflected by differences in K_{f} (Fig. 2), was even more profound in patients with renal failure (5 ± 1 vs. 25 ± 3 in SSA controls, P < 0.05). According to equation 1, when pressure in the glomerular capillaries rises, the ultrafiltration coefficient has to fall, if GFR does not change. Consequently, if transglomerular hydraulic pressure (ΔP) were 40 mm Hg in patients with glomerular disease, then the albuminuria group would have an ultrafiltration coefficient, K_{f}, of only 50% of that of SSA controls. For the CRF group, K_{f} would only be 15% of that of SSA controls (Fig. 2).

Both albumin and IgG excretion rates were inversely related to GFR (r = −0.61 and r = −0.69, respectively, P < 0.001). The correlations were stronger when more specific markers of glomerular injury (ultrafiltration coefficient and fractional protein clearances) were considered (Fig. 3). Glomerular permeability, as assessed by the fractional clearances of albumin (Fig. 3A) and IgG (Fig. 3B) was correlated inversely with the ultrafiltration coefficient (r = −0.68 and r = −0.74, respectively, P < 0.001). Thus, as ultrafiltration coefficient is reduced, glomerular permeability (enhanced fractional clearances of albumin and IgG) is also lost.

**Discussion**

We applied physiological techniques in a cross section of subjects with sickle cell anemia to examine the course of renal insufficiency and glomerular injury in patients with sickle cell nephropathy. We found that renal insufficiency occurs primarily because of a decrease in the ultrafiltration capacity (reflected in the calculated ultrafiltration coefficient), along with a reduction in

### Table 1. Clinical features

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Gender (M/F)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Hematocrit (%)</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>S. creatinine (mg/dl)</th>
<th>Fractional creatinine clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA controls (N = 10)</td>
<td>30 (23-43)</td>
<td>3/7</td>
<td>70 ± 5</td>
<td>166 ± 4</td>
<td>30 ± 2</td>
<td>120 ± 5</td>
<td>63 ± 5</td>
<td>0.7 ± 0.1</td>
<td>1.40 ± 0.08</td>
</tr>
<tr>
<td>Albuminuria (N = 7)</td>
<td>27 (23-60)</td>
<td>5/2</td>
<td>65 ± 6</td>
<td>170 ± 4</td>
<td>31 ± 3</td>
<td>124 ± 7</td>
<td>73 ± 8</td>
<td>0.7 ± 0.1</td>
<td>1.37 ± 0.06</td>
</tr>
<tr>
<td>CRF (N = 17)</td>
<td>36 (18-65)</td>
<td>7/10</td>
<td>60 ± 3</td>
<td>165 ± 3</td>
<td>21 ± 1°C</td>
<td>126 ± 4</td>
<td>71 ± 4</td>
<td>1.5 ± 0.3</td>
<td>1.69 ± 0.11</td>
</tr>
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</table>

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure.

* Median (range)

**P < 0.05 vs. SSA controls

### Table 2. Serum proteins and excretion

<table>
<thead>
<tr>
<th></th>
<th>S. albumin (g/liter)</th>
<th>S. IgG (mg/dl)</th>
<th>AER (μg/min)</th>
<th>IgG ER (mg/min)</th>
<th>Fractional albumin clearance (× 10^{-5})</th>
<th>Fractional IgG clearance (× 10^{-5})</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA controls</td>
<td>4.9 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>17 ± 6</td>
<td>3 ± 1</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>4.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>605 ± 195a</td>
<td>32 ± 15a</td>
<td>11 ± 3a</td>
<td>2 ± 1a</td>
</tr>
<tr>
<td>CRF</td>
<td>3.9 ± 0.6</td>
<td>1.5 ± 0.1</td>
<td>868 ± 206a</td>
<td>171 ± 111a</td>
<td>114 ± 47ab</td>
<td>58 ± 41ab</td>
</tr>
</tbody>
</table>

Abbreviations: AER, albumin excretion rate; IgG ER, IgG excretion rate.

* P < 0.05 vs. SSA controls; ** P < 0.05 vs. albuminuria

### Table 3. Glomerular dynamics

<table>
<thead>
<tr>
<th></th>
<th>MAP (mm Hg)</th>
<th>GFR (ml/min/1.73)</th>
<th>RPF</th>
<th>FF</th>
<th>K_{f}</th>
<th>ΔP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA controls</td>
<td>82 ± 5</td>
<td>112 ± 7</td>
<td>734 ± 75</td>
<td>0.16 ± 0.01</td>
<td>25 ± 3</td>
<td>35</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>90 ± 7</td>
<td>97 ± 5</td>
<td>618 ± 55</td>
<td>0.16 ± 0.01</td>
<td>16 ± 3a</td>
<td>35</td>
</tr>
<tr>
<td>CRF</td>
<td>89 ± 4</td>
<td>44 ± 5ab</td>
<td>366 ± 51ab 0.13 ± 0.01</td>
<td>5 ± 1ab</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MAP, mean arterial pressure; GFR, glomerular filtration rate; FF, filtration fraction; RPF, renal plasma flow; K_{f}, ultrafiltration coefficient (ml/(min · mm Hg/1.73)); ΔP, transglomerular capillary hydraulic pressure.

* P < 0.05 vs. SSA controls; ** P < 0.05 vs. albuminuria
renal plasma flow. When albumin and IgG excretion are abnormally high, glomerular damage is present, since the ultrafiltration coefficient is reduced, compared to non-proteinuric SSA patients. This also occurs in albuminuric patients with a normal value of GFR. If intraglomerular pressures were high in those patients with glomerular injury, the reduction of ultrafiltration coefficient would be even more pronounced (Fig. 2). Moreover, the ultrafiltration coefficient was inversely correlated with fractional protein clearances, also suggesting the presence of glomerular capillary wall injury (Fig. 3). In short, our analysis indicates that loss of glomerular permselectivity is accompanied by a striking loss of ultrafiltration coefficient. This finding suggests that a continuum of glomerular injury occurs in SSA, but documenting this in a longitudinal study could take up to 10 to 15 years (Fig. 1). The injury is already present in patients with modest albuminuria but a normal GFR, and is more severe in patients with overt renal insufficiency. It is worth noting that a significant loss of glomerular function could not be documented simply by measuring GFR, but a detailed hemodynamic analysis of glomerular function uncovers the severity of the damage.

To characterize glomerular permselectivity [22], we measured the absolute excretion and fractional clearance of two proteins of different size and electrical charge: IgG (a large uncharged protein) and albumin (a smaller, negatively-charged protein). High values of IgG excretion in the albuminuria and CRF groups (Table 2) suggest that size-selectivity is lost early in the course of sickle cell glomerulopathy. Whether charge-selectivity is also impaired, as suggested by the higher fractional clearance of albumin than IgG (Table 2), cannot be assessed from the present study, because of the unknown contribution of variation in tubular protein reabsorption [31].

What could account for these findings? In children with SSA, glomerular function is characterized by increased blood flow and GFR, and a low filtration fraction [10]. Recently, glomerular hypertrophy was described in young adults with SSA who had no overt renal abnormalities [32]. While glomerular hypertrophy could account for a high GFR in SSA controls, by itself, it cannot explain the disproportionately higher renal plasma flow present in patients with SSA (signified by the filtration fraction of 0.16 in SSA controls vs. a filtration fraction of 0.20 in healthy non-SSA individuals, data not shown). This result suggests renal vasodilation. In experimental animals, creation of acute, isovolemic anemia is accompanied by marked increase in renal blood flow and an increase [33] or no change in GFR [34]. Filtration fraction is always low. In micropuncture studies, acute anemia is associated with a marked reduction in pregglomerular resistance, which explains the increase in glomerular blood flow. However, there is no increase in the pressure within the glomerular capillaries [34]. So, renal vasodilatation related to anemia leading to an increase in glomerular blood flow could mask losses in ultrafiltration coefficient and maintain GFR (Table 3).

The ultrafiltration coefficient, \( K_f \), is the product of the total capillary area available for filtration (S) and the intrinsic hydraulic conductivity of the glomerular capillary wall (k). Alterations in capillary filtering area (that is, early global or segmental sclerosis) or intrinsic hydraulic conductivity (that is, following glomerular capillary wall injury) or a combination of both could account for the reduction in ultrafiltration coefficient we found in SSA patients with proteinuria. Recently, Falk et al described the glomerular lesions in SSA patients with similarities to the CRF group we studied (the median level of proteinuria in their study was 1.4 g/day and mean GFR was 78 ml/min). The predominant lesions they described include focal segmental and global glomerulosclerosis and glomerular hypertrophy [7]. These lesions as well as mesangial proliferation with reduplication of the glomerular basement membrane and sclerosis which have been found in nephrotic SSA patients [15-17] could cause the functional glomerular abnormalities we found.

The two-kidney RPF was reduced in the CRF group as compared to controls (Table 3). Such a reduction could independently of \( K_f \) contribute to a reduction in GFR in the CRF group. However, based on the reported pathology in patients with SSA and renal insufficiency [7], it is likely that our CRF patients have significant global sclerosis and, thus, reduced total nephron number. Such nephron loss could account for the reduction in two-kidney RPF we found in the CRF group. Thus, the fall in RPF in the latter group could be secondary to a loss of glomeruli and not to a reduction in single nephron RPF.

The prevalence of renal insufficiency in SSA has been estimated at 7%, based on high values of serum creatinine [7]. At the Emory Sickle Cell Center, only 21 of 342 patients (6%) had serum creatinine in excess of 1.4 mg/dl (unpublished observations). We conclude that the prevalence of glomerular damage in SSA patients is undoubtedly greater. As shown in Figure 1, serum creatinine is a poor marker of glomerular function in patients with SSA; 17 of the 34 patients we studied (50%) had subnormal GFR and 24 (70%) had glomerular dysfunction signified as reduced ultrafiltration coefficient and abnormal glomerular permselectivity. Despite these findings, serum creatinine was elevated in only 5 of the 34 patients. Unfortunately, we cannot document the true prevalence of glomerular abnormalities in SSA, since patients with established renal dysfunction may be more likely to participate in a study of glomerular function. The true prevalence of renal failure is also difficult to estimate. According to the most
recent USRDS report, in the period 1989 to 1992, only 206 patients with SSA were receiving treatment for ESRD in the U.S. [35]. The fact that this number is smaller than expected could be explained by the high mortality of these patients. In patients with SSA, renal failure is a strong predictor of early death: the median life expectancy of SSA patients with renal failure is 20 years less than that of patients without renal insufficiency [8]. Once on dialysis, the survival of SSA patients is also poor: at 20 months only 40% are alive [36]. Thus, we suspect that an excess mortality may account for the apparent low prevalence of SSA in ESRD programs.

It is not known whether SSA patients with albuminuria will progress to renal insufficiency, but based on longitudinal studies of other glomerular diseases [37, 38], such patients are at high risk for progression. Recently, converting enzyme inhibition has been shown to reduce proteinuria acutely in SSA patients with renal insufficiency [7] and in those with microalbuminuria and normal creatinine clearance [39]. Whether these effects are related solely to a reduction in intraglomerular pressure is not known, nor is it known whether a reduction in proteinuria will change the progression of renal insufficiency.

Screening for proteinuria is usually performed by urine dipstick. Because adult SSA patients are hypo-isosthenuric and are encouraged to drink large volumes of fluids [40], dilute urine could hamper the detection of early glomerular abnormalities. Measurement of GFR and its hemodynamic determinants are available only as a research tool, but albumin or IgG excretion can be measured using sensitive techniques (that is, radioimmunoassay) and thus, these measurements have the potential of detecting early glomerular injury in patients with hemoglobin SS or SC. At this stage, glomerular injury may be more amenable to therapeutic intervention.

Acknowledgments

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References


