Medullary Microvascular Thrombosis and Injury in Sickle Hemoglobin C Disease

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ABSTRACT

Sickle cell nephropathy is a common complication in patients with sickle cell hemoglobinopathies. In these disorders, polymerization of mutated hemoglobin S results in deformation of red blood cells, which can cause endothelial cell injury in the kidney that may lead to thrombus formation when severe or manifest by multilayering of the basement membranes (glomerular and/or peritubular capillaries) in milder forms of injury. As the injury progresses, the subsequent ischemia, tubular dysfunction, and glomerular scarring can result in CKD or ESRD. Sickle cell nephropathy can occur in patients with homozygous hemoglobin SS or heterozygous hemoglobin S (hemoglobin SC, hemoglobin S/β0-thalassemia, and hemoglobin S/β+–thalassemia). Clinical manifestations resulting from hemoglobin S polymerization are often milder in patients with heterozygous hemoglobin S. These patients may not present with clinically apparent acute sickle cell crises, but these milder forms can provide a unique view of the kidney injury in sickle cell disease. Here, we report a patient with hemoglobin SC disease who showed peritubular capillary and vasa recta thrombi and capillary basement membrane alterations primarily involving the renal medulla. This patient highlights the vascular occlusion and endothelial cell injury in the medulla that contribute to sickle cell nephropathy.


A 42-year-old black woman was diagnosed with hemoglobin (Hb) SC disease at 8 months of age. She experienced many sickle cell crises when she was younger. Her last crisis at age 20 years old involved a cerebrovascular accident that was complicated by several subsequent seizures, and she later developed sickle cell retinopathy. Her last Hb electrophoresis at age 26 years old showed 46.8% HbS and 41.6% HbC. At age 34 years old, her serum creatinine was 0.5–0.8 mg/dl and steadily increased to 1.1 (age 36 years old), 1.3 (age 37 years old), 1.5 (age 38 years old), and 1.7 mg/dl (now). The 24-hour urine protein collection was 1.25 g. Urinalysis showed 1+ blood. Serologic workup showed positive antinuclear antibodies (titer 1:320), but antidual–stranded DNA antibodies and antineutrophil cytoplasmic antibody titers were negative, and complement levels were normal. A kidney biopsy was performed.

KIDNEY BIOPSY

The light microscopic sample contained 20 glomeruli, of which nine glomeruli were globally sclerosed and two glomeruli were segmentally sclerosed. Some of the remaining glomeruli showed rare duplication of the glomerular basement membranes without significant mesangial or endocapillary hypercellularity. Approximately 20% of the glomeruli were hypertrophic. Many medullary peritubular capillaries (PTCs) were congested with sickle–shaped red blood cells (RBCs), and some PTCs (Figure 1) and vasa recta (Figure 2) contained thrombi with RBC fragments that completely occluded the lumen, which contrasted with the absence of these findings in the cortex. Compared with the patchy mild interstitial fibrosis and tubular atrophy in the cortex, the medulla showed focally marked tubular atrophy, interstitial fibrosis, and PTC loss. Hemosiderin was not noted in the tubules, and a Prussian blue stain for iron was negative.

Immunofluorescence microscopy (scale of 0–4+) of six glomeruli (three globally sclerotic) showed granular mesangial staining for IgA (2+), C3 (2+), and κ–λ (1+) and A-light chains (1–2+). There was no glomerular staining for IgG, IgM, C1q, fibrinogen, or albumin. No significant tubular basement membrane or vascular staining was seen.

Ultrastructural evaluation showed irregularly shaped RBCs in the medullary PTCs, with numerous cytoplasmic fibrils (Figure 3) that were consistent with polymerized Hb. In contrast, RBCs in the glomeruli and cortical PTCs were normal.
There was frequent endothelial cell swelling in the medullary PTCs and glomerular capillaries. There was multilayering of the medullary PTC basement membranes (Figure 4) and rare duplication of glomerular basement membranes, whereas the cortical PTCs did not show basement membrane alterations. Occasional mesangial immune–type electron dense deposits were also present.

The final diagnosis was sickle cell nephropathy (SCN) with PTC and vasa recta thrombi and capillary basement membrane alterations along with a minor component of IgA glomerular deposition.

**CLINICAL FOLLOW-UP**

She was initially started on lisinopril for the proteinuria, but this was discontinued because of a drop in Hb (6.4 g/dl; baseline: 8 g/dl). She was then transitioned to losartan (25 mg), and her spot urine protein-to-creatinine ratio has dropped to 0.91 g since initiation of the angiotensin receptor blocker. Her serum creatinine has remained between 1.6 and 1.8 mg/dl.

**DISCUSSION**

Sickle cell hemoglobinopathies (or sickle cell disease) are inherited disorders involving a single-point mutation in the gene that encodes β-globulin. This point mutation at the sixth amino acid in β-globulin causes valine to substitute for glutamic acid and produces sickle Hb (HbS).\(^1\,^2\) This substitution results in exposure of a hydrophobic motif in the deoxygenated form of HbS that causes binding of Hb chains and HbS polymerization. The degree of disease severity is related to the rate and extent of HbS polymerization. Homozygosity for this mutated form of Hb, HbSS, results in the most severe form of disease (also known as sickle cell anemia). Heterozygosity for HbS produces disease with varying and intermediate severity. These heterozygous disorders include HbSC and HbS/β-thalassemias. HbC involves a substitution of lysine for glutamic acid in β-globulin and causes HbC to be less soluble than normal Hb. This mutation does not cause HbC polymerization; however, HbC can enhance HbS polymerization through red cell dehydration. Although HbSC disease is milder than...
HbSS, acute chest syndrome, retinopathy, and osteonecrosis can still occur. HbS/β-thalassemias includes several types (including HbS/β°-thalassemia and HbS/β+-thalassemia) on the basis of the β-thalassemia mutation. The disorder in patients with sickle cell trait (HbAS) is the least severe.

SCN is a common complication of sickle cell hemoglobinopathies, where 30%–68% of adults with sickle cell hemoglobinopathies develop renal dysfunction. CKD is more prevalent in adults with HbSS than HbSC, and patients with HbSC account for about one third of patients with SCN. SCN results from vaso-occlusion by sickled RBCs, which preferentially occurs in the medulla, because this region of the kidney is relatively hypoxic. The acidic environment and the hyperosmolality of the medulla also increase the intracellular Hb concentration and decrease HbS affinity for oxygen. These factors promote HbS polymerization, which can be observed ultrastructurally in the cytoplasm of abnormal RBCs (Figure 3). Polymerization of HbS in the RBCs alters their shape and slows blood flow, which can cause injury and RBC adhesion to endothelial cells and ultimately, PTC thrombi. The impaired blood flow results in ischemia, microinfarcts, and when severe, papillary necrosis. Over time, PTC loss and interstitial fibrosis will result, which was also observed in our patient. Clinically, these features cause hematuria and tubular defects, including impaired potassium excretion and urine concentrating defects, or hyposthenuria.

In contrast to hypoperfusion of the medulla, there is hyperperfusion of the cortex, and both can occur concurrently. Cortical hyperperfusion causes glomerular hypertrophy, and these changes can occur early in childhood in otherwise asymptomatic patients. The cause of this cortical hyperperfusion is not fully understood. Hyperperfusion may be reflective of the systemic decrease in vascular resistance in SCD, and many patients with sickle cell have lower BPs before onset of kidney disease. Reduction of vasculature in the renal medulla from thrombosis could lead to more blood flow to the cortex, and the subsequent hypoperfusion of the medulla could result in distal nephron dysfunction and compensation of the proximal tubules. Prostaglandin release from worsening hypoxia and nitric oxide depletion may be additional contributing factors by decreasing vascular resistance, but endothelial activation, nitric oxide deficiency, and ischemia-reperfusion injury have also been suggested to contribute to the pathophysiology.

In our patient, the medullary PTCs also commonly show basement membrane multilayering, which is similar to
chronic antibody–mediated rejection and has not been previously reported in SCN. Multilayering of the PTC basement membranes can occur in the native kidney. Drachenberg et al. were among the first investigators to identify significant PTC basement membrane alterations in seven (8%) of 85 native kidney biopsies. Ivanyi et al. observed PTC duplication in three (6%) of 56 native kidney disease biopsies: one biopsy with chronic tubulointerstitial nephritis and two biopsies with thrombotic microangiopathy. These findings were confirmed by Gough et al., who found PTC basement membrane duplication in 13 (9%) of 143 native kidney biopsies, including lupus nephritis, pauci-immune crescentic GN, malignant nephrosclerosis, thrombotic microangiopathy (one biopsy with antiphospholipid antibodies), cocaine–associated acute tubular necrosis, fibrillary GN, Henoch–Schönlein purpura nephritis, and minimal change disease. In contrast, Liapis et al. recently found PTC multilamination in 76% of native kidney biopsies with three or fewer layers but only 6% with five or six layers. To our knowledge, our study is the first to describe PTC alterations in SCN, and we suspect that this may be a common pathologic feature of this disease that has been underappreciated.

In summary, kidney biopsies of the milder forms of SCN, such as in this patient, provide important opportunities to observe the disease. Our study highlights the central role of microvascular thrombosis and endothelial injury in the disease progression of SCN. In particular, medullary PTC and vasa recta alterations may provide the only diagnostic clues to the underlying etiology of sickle cell disease, which can be easily overlooked during the pathologic evaluation.

DISCLOSURES
A.C. is on the speaker bureau for Alexion Pharmaceuticals.

REFERENCES

3. Bunn HF, Noguchi CT, Hofrichter J, Schechter GP, Schechter AN, Eaton WA: Molecular and cellular pathogenesis of hemoglobin ischemic events can result in chronic changes in the medulla and contribute to SCN.

A variety of glomerular lesions occurs in the setting of SCN, including global glomerulosclerosis, FSGS (often the perihilar variant and rarely the collapsing variant), and membranoproliferative GN. Moderate IgA glomerular deposition was present in our patient, and it has been rarely reported in patients with HbSS and HbSC. Although a coincidental finding of IgA nephropathy is possible, given that it is the most common GN worldwide, liver injury caused by sickle cell hemoglobinopathy occurs, and hepatic glomerulosclerosis (or secondary IgA nephropathy) in the setting of portal hypertension or cirrhosis may be an alternate explanation. However, our patient did not manifest hepatomegaly or any clinical laboratory findings that suggested the presence of liver disease. Also, this distinction may be blurred, because abnormal glycosylation of IgA is observed in both settings. In either scenario, the glomerular alterations were a minor aspect of the kidney biopsy findings.

Figure 4. Multilayering of PTC basement membranes in the medulla. This PTC in the medulla shows multilayering of the basement membranes, which indicates repetitive endothelial cell injury. Scale bar, 1 μm.