KIDNEY BIOPSY TEACHING CASE

New-Onset Proteinuria With Massive Amorphous Glomerular Deposits

Dylan V. Miller, MD, Ahmet Dogan, MD, PhD, and Sanjeev Sethi, MD, PhD

INDEX WORDS: Amyloid, hereditary; fibrinogen A α chain; glomerular disease.

We present 2 patients with long-standing mild hypertension who presented with new-onset proteinuria and decreased kidney function. We describe interesting and unusual kidney biopsy findings in these 2 patients.

CASE REPORTS

Clinical History and Initial Laboratory Data

Case 1

A 57-year-old man presented with nephrotic syndrome and decreased kidney function. Serum creatinine level was 2.0 mg/dL (177 μmol/L, corresponding to an estimated glomerular filtration rate of 57 mL/min/1.73 m^2, assessed by using the 6-variable Modification of Diet in Renal Disease [MDRD] Study equation), and 24-hour urinary protein excretion was 10.2 g. He was previously in good health, and his medical history included mild hypertension, for which he was treated with β-blockade. There was no history of autoimmune conditions or other systemic diseases. His mother and grandmother had both been given a diagnosis of Bright disease, and his brother also had proteinuria. Serological test results for antinuclear, anti–double-stranded DNA, antineutrophil cytoplasmic, and anti–glomerular basement membrane antibodies were negative. No monoclonal protein was detected by means of serum and urine protein electrophoresis and immunofixation studies. No antibodies to hepatitis B and C virus antigens were detected in serum. Kidney biopsy was performed to determine the cause of proteinuria and decreased kidney function.

Case 2

A 59-year-old man presented with shortness of breath and wheezing that did not improve with antibiotic therapy. He was found to have decreased kidney function (serum creatinine, 8.2 mg/dL [724 μmol/L], corresponding to an estimated glomerular filtration rate $<$ 10 mL/mL/1.73 m^2), and a workup for pulmonary-renal syndrome ensued. Urinalysis with microscopy did not show active sediment. Antineutrophil cytoplasmic antibody test results were negative. There was no hemoptysis or significant pulmonary disease. He also was in good health before this and likewise had long-standing mild hypertension. There was no history of autoimmunity. He did not have significant nonsteroidal anti-inflammatory drug use. Family history was negative for abnormal urinalysis results and kidney failure. Kidney biopsy was performed to determine the cause of decreased kidney function.

Laboratory findings at presentation for both patients are listed in Table 1.

Kidney Biopsy

In both patients, a kidney biopsy was performed, yielding adequate samples. There were more than 15 nonsclerotic glomeruli in the light microscopy sample. Every glomerulus showed massive mesangial expansion and thickening of the peripheral capillary loops (Figs 1 and 2), resulting in almost complete occlusion of the capillary lumen. The expansion was mostly acellular, resulting in large glomeruli filled with amorphous material with almost no discernable capillary lumen. The matrix material showed relatively negative staining by means of Jones methenamine–silver and periodic acid–Schiff (PAS) stains. Amyloid "spicules" were not seen. The material in capillary walls and loops appeared identical to that in the mesangium. The tubulointerstitial compartment showed moderately advanced fibrosis and tubular atrophy in a patchy distribution. Muscular arteries and arterioles showed mild sclerosis of the intima, but no morphological evidence of amyloid deposition.

Congo Red staining was strongly positive in glomeruli and showed the appropriate birefringence under plane-polarized light and light emission under the fluorescence microscope. Congo Red staining was negative in the interstitium and vessels.

Immunofluorescence in each patient showed at most weak (1+ to 2+) "smudgy" glomerular staining for immunoglobulin G (in patient 2) and fibrinogen (in patient 1; Fig 1D). No significant staining was seen for immunoglobulin A, immunoglobulin M, C1q, C3, or fibrinogen. Staining for κ and λ immunoglobulin light chains was polytypic and weak in each patient.

By means of electron microscopy, changes in each biopsy specimen were essentially identical. There was extensive mesangial widening and deposition of a flocculent material that on greater magnification was composed of nonbranching randomly oriented fibrils with a diameter of 10 to 12 nm. This same material was seen expanding the peripheral capillary walls in a global fashion. Deposits were not organized into spicular structures, and there was no significant basement membrane reaction. In patient 2, there was focal

From the Division of Anatomic Pathology, Mayo Clinic, Rochester, MN.

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Address correspondence to Sanjeev Sethi, MD, PhD, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. E-mail: sethi.sanjeev@mayo.edu

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Table 1. Laboratory Findings

	Patient 1	Patient 2
Serum creatinine (mg/dL)	2.0	8.2
Estimated glomerular filtration rate (mL/min/1.73 m^2)	57	<10
Total protein (g/dL)	5.7	—
Serum albumin (g/dL)	3.6	4.2
Hemoglobin (g/dL)	13.0	10.9
Hematocrit (%)	39.5	33.3
Urinary protein (g/24 h)	10.2	*
Additional studies	Monoclonal protein	No monoclonal protein

Note: Glomerular filtration rate was estimated by using the 6-variable Modification of Diet in Renal Disease Study equation. Conversion factors for units: serum creatinine in mg/dL to mmol/L, ×88.4, estimated glomerular filtration rate in mL/min/1.73 m^2 to mL/s/1.73 m^2, ×0.01667; hemoglobin in g/dL to mmol/L, ×10, total protein and albumin in g/dL to g/L, ×10.

*The 24-hour quantitation was not performed for this oliguric patient; however, urine dipstick showed proteinuria (3+).

deposition of the fibrillary material within an arteriole on ultrastructural examination.

Because immunofluorescence studies were negative for AL amyloid, we performed immunohistochemical studies to determine whether the amyloid was secondary (AA) amyloid. Glomeruli stained for serum amyloid protein (SAP), but were negative for AA amyloid, suggesting an uncommon type of amyloid.

To further classify/identify the amyloid, we next performed laser capture microdissection and mass spectrometry (MS) analysis.[1] Congo Red–positive regions of glomeruli in each case were microdissected by using laser capture techniques. Peptides extracted from the microdissected tissue were subjected to liquid chromatography and tandem MS (LC-MS/MS). In both patients' samples, the most abundant peptides detected represented serum amyloid P component, fibrinogen α chain, and apolipoprotein E proteins and their precursors. LC-MS/MS failed to detect peptides representing transthyretin, serum amyloid A component, or immunoglobulin light chains. The findings are shown in Fig 3.

Diagnosis

Amyloidosis, fibrinogen α chain (AFib) type.

Clinical Follow-up

Patient 1

At 3 months after the kidney biopsy, this patient's serum creatinine level was stable, but increased (1.9 mg/dL [168 μmol/L]). On angiotensin-converting enzyme inhibition therapy, as well as fish oil supplementation, proteinuria had decreased slightly (protein, 5 g/24 h).

Patient 2

At 9 months after the kidney biopsy, this patient remained on dialysis therapy, with a serum creatinine level of 13.4 mg/dL (1,185 μmol/L). He is being considered for combined liver-kidney transplantation.

DISCUSSION

These 2 fascinating cases of patients with a rare heredofamilial form of amyloidosis with long-standing hypertension and decreased kidney function are similar in several respects. Kidney biopsy findings in both patients showed massive acellular nodular glomerular expansion with silver-negative and PAS-negative material.

The differential diagnosis of nodular glomerulosclerosis includes thrombotic microangiopathy; healed membranoproliferative glomerulonephritis; unusual diabetic nodules; deposition diseases, including amyloid or light chain deposition disease; and such rare causes as collagen glomerulopathy or crystal storing histiocytosis. In both patients described, vessels were unremarkable, making a diagnosis of thrombotic microangiopathy unlikely. Membranoproliferative glomerulonephritis was excluded because the mesangial expansion was acellular, double contours were not present, and immunofluorescence was negative for immune deposits. Diabetic nodules are PAS and silver positive, whereas nodules in the 2 patients were essentially silver and PAS negative. Finally, light-chain deposition was excluded because glomerular and tubular basement membranes were not positive for either light chain on immunofluorescence studies. Collagen glomerulopathy was high on the differential diagnosis. However, the positive Congo Red staining of glomeruli and electron microscopy studies showing fibrils measuring 8 to 10 nm in diameter confirmed the diagnosis of amyloidosis.

The diagnosis of amyloidosis usually is based on positive Congo Red and/or Thioflavin T stains. Routine subtyping of amyloidosis into AL amyloidosis (light chain–associated amyloid) and AA amyloidosis (secondary amyloid) then is carried out by staining for λ and κ light chains and stains for SAP component and serum amyloid A protein. In AL amyloid, amyloid stains for SAP and either λ or κ light chains. In cases of AA amyloidosis, amyloid stains for SAP and serum amyloid A, but is