INTERNAL MEDICINE

□ CASE REPORT □

Light Chain Deposition Disease Diagnosed with Laser Micro-dissection, Liquid Chromatography, and Tandem Mass Spectrometry of Nodular Glomerular Lesions

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Abstract

A 42-year-old man developed nephrotic syndrome and rapidly progressive renal failure. Kidney biopsy demonstrated nodular glomerulosclerosis, negative Congo red staining, and no deposition of light or heavy chains. Laser micro-dissection and liquid chromatography with tandem mass spectrometry of nodular lesions revealed the presence of a kappa chain constant region and kappa III variable region, which signified light chain deposition disease. Dexamethasone and thalidomide were effective in decreasing the serum levels of free kappa light chain from 147.0 to 38.0 mg/L, eliminating proteinuria, and halting the worsening of the kidney dysfunction, with serum creatinine levels stable around 4.0 mg/dL for 3 years.

Key words: nodular glomerulosclerosis, light chain deposition disease, microfibril, mass spectrometry

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Introduction

Light chain deposition disease (LCDD) has an incidence of approximately 0.33-0.5% in kidney biopsy specimens (1-3) and is characterized by the deposition of monoclonal light chains in systemic tissues and negative Congo red staining. The diagnosis of LCDD can be easily made with kidney biopsy, as the kidney is usually the affected organ, with manifestations such as proteinuria and decreased kidney function. The characteristic morphological findings of LCDD are nodular glomerulosclerosis and nonfibrillar electron-dense deposits on the glomerular or tubular basement membrane seen with electron microscopy.

We herein present a case of nodular glomerulosclerosis with nephrotic syndrome and rapidly progressive renal failure, which was initially compatible with idiopathic nodular glomerulosclerosis (ING). The final diagnosis of LCDD (kappa chain constant region and kappa III variable region) was made with laser micro-dissection and liquid chromatography with tandem mass spectrometry (LC-MS/MS) of nodular glomerular lesions. We also discuss the treatment for LCDD.

Case Report

A 42-year-old Japanese man was admitted to Aichi Medical University Hospital because of nephrotic syndrome and progressive renal failure. Six months prior to admission, he visited a local hospital for evaluation of bilateral lower extremity edema. He was diagnosed with nephrotic syndrome and progressive renal failure based on data such as a urinary protein level of 3.94 g/g·Cr, serum albumin level of 2.8 g/dL, and serum creatinine level of 2.36 mg/dL. Kidney biopsy at the previous hospital demonstrated nodular glomerulosclerosis in all 29 glomeruli examined (Fig. 1A and B). Deposition was also found in the walls of the small arterioles, but not in the tubular basement membrane or interstitial area. Electron microscopy showed randomly arranged fibrillar structures with a width of 7-13 nm in the subendo-

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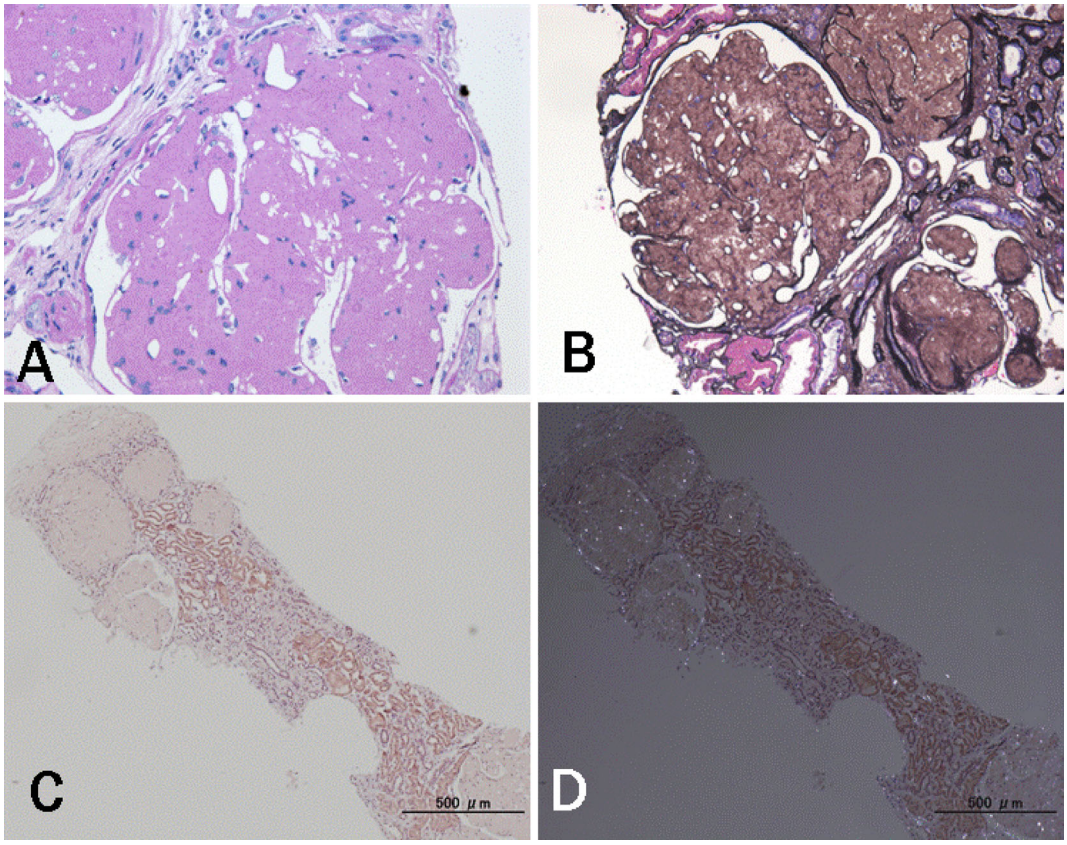

Figure 1. **The light microscopy findings of the kidney biopsy specimen. (A) Nodular glomerulosclerosis was observed in the glomeruli, PAS ×400. (B) Massive deposition and a nodular pattern in glomeruli, PAS ×200. (C) Negative Congo red staining, Congo red ×50. (D) Negative green birefringence under polarized light with Congo red staining, Congo red ×50.**

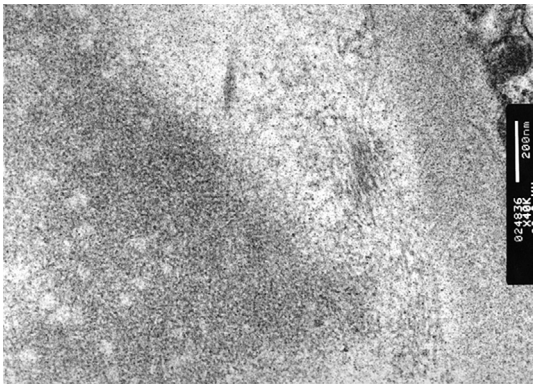

Figure 2. **The electron microscopy findings. Randomly arranged fibrillar structures with a width of 7-13 nm were observed in the subendothelial and mesangial areas. Bar, 200 nm.**

thelial and mesangial areas (Fig. 2); however, electron-dense deposits were not observed. Congo red staining was negative at the previous hospital; there was very weak positivity and no characteristic green birefringence under polarized light (Fig. 1C and D). Immunofluorescence studies were negative for IgG, IgA, IgM, and C3. Unfortunately, staining for kappa and lambda light chains was not performed. Oral prednisolone (20 mg/day) was started for ING. The proteinuria improved gradually; however, his kidney function worsened at six months after onset. The patient was referred to our hospital. He does not smoke, and his medical history and family history were deemed non-contributory.

On admission, his height was 173 cm, body weight 61.0 kg, body temperature 36.8℃, blood pressure 128/68 mmHg, pulse 68 beats/min, and SpO_2 98% on room air. A physical examination revealed bilateral lower extremity edema. No macroglossia, lymphoadenopathy, or eruptions were observed. The laboratory studies indicated 2^+ proteinuria (1.49 g/day), no occult hematuria, a white blood cell count of 7,900, a red blood cell count of 415×10^4/μL, hemoglobin of 13.3 g/L, hematocrit of 40.9%, a platelet count of 18.8×10^4/μL, albumin level of 3.6 g/dL, blood urea nitrogen level of 29.4 mg/dL, serum creatinine level of 4.43 mg/dL, and total cholesterol level of 196 mg/dL. His Na level was 146 mEq/L, K level was 4.3 mEq/L, Cl level was 108 mEq/L, AST level was 15 U/L, and ALT level was 15 U/L. His lactate dehydrogenase level was 235 IU/L, CK level was 52 IU/L, and CRP level was 0.10 mg/dL. His IgG, IgA, and IgM antibody levels were 437 mg/dL, 43 mg/dL, and 99 mg/dL, respectively. His complement C3 level was 77.8 mg/dL (normal range, 60-120 mg/dL), C4 level was 19.2 mg/dL (normal range, 14-40 mg/dL), and CH50 level was 35.5 U/mL (normal range, 30-40 U/mL). The findings for rheumatoid factor, anti-nuclear antibody, hepatitis B antigen, hepatitis C

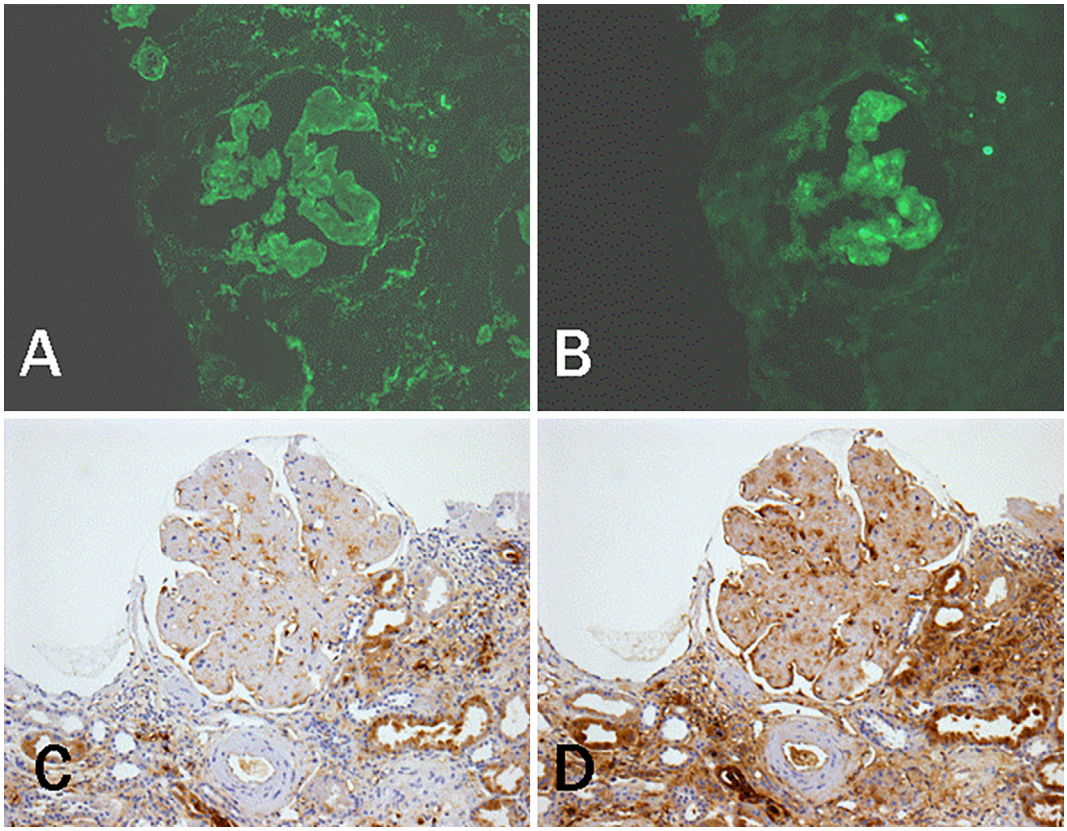

Figure 3. **Immunofluorescence and immunohistochemical studies demonstrated no significant deposition of kappa and lambda light chains. (A) The non-specific findings in the nodular lesions, kappa chain immunofluorescence study, ×200. (B) Small spots were observed in the subendothelial spaces. There were non-specific findings in the nodular lesions in the lambda chain immunofluorescence study, ×200. (C) Negative findings in the nodular lesions, kappa chain immunohistochemical study, ×200. (D) Negative findings in the nodular lesions, lambda chain immunohistochemical study, ×200.**

virus antibody, and other autoantibodies were all negative. Immunoelectrophoresis and immunofixation of serum and urine showed no M-proteins. Free kappa and lambda light chain levels were 147.0 and 15.0 mg/L, respectively. His NT-proBNP level was 771 pg/mL (normal range, below 125 pg/mL), and his P-III-P level was 0.84 U/mL (normal range, 0.3-0.8 U/mL). These data suggested partial remission of nephrotic syndrome with oral prednisolone therapy.

Electrocardiography demonstrated low voltage in the limb leads, and transthoracic echocardiography showed a thickened left ventricle wall and interventricular septum (12 mm). Bone marrow aspiration revealed 1.8% plasma cells, and immunostaining and flow cytometry of marrow cells demonstrated no kappa or lambda deflection. Biopsy of adipose tissue from the abdominal wall and random biopsies of lower and upper gastrointestinal tract mucous membranes revealed no immunoglobulin or amyloid deposition. We re-examined the kidney biopsy specimens to clarify the deposition of amyloid and light chains. Congo red staining was negative at our institute and Kumamoto University. Immunofluorescence and immunohistochemical studies demonstrated no significant deposition of immunoglobulin heavy or light chains (Fig. 3) or fibronectin. These findings were consistent with idiopathic glomerulosclerosis.

Laser micro-dissection and LC-MS/MS of the nodular glomerular lesions revealed substantial deposition of the kappa chain constant region and kappa chain III variable region (Fig. 4). However, the level of amyloid P component was very low as a background score. We made a diagnosis of LCDD. He did not have monoclonal proteins in the serum or urine by immunoelectrophoresis and immunofixation, only a predominant free kappa chain in the serum.

After admission, we changed his prednisolone therapy to dexamethasone (Dex) therapy (40 mg/day for 4 days each month), based on the guidelines for multiple myeloma (4), even though prednisolone had been somewhat effective, as evidenced by the gradual decreases in the urinary protein levels. Six months after initiating Dex therapy, his serum levels of free kappa light chain decreased from 147 to 63.7 mg/L, and the kappa/lambda ratio was 2.61. At 12 months after admission, we added 50 mg/day of oral thalidomide (Thal). Thal-Dex therapy was effective in this patient; the serum levels of free kappa light chain decreased to 38 mg/L, his proteinuria resolved (less than 0.3 g/day), and there was no progressive decline in his kidney function, with serum creatinine levels stable around 4.0 mg/dL for 3 years

Accession	Description	%	
P01834	IgG kappa chain C region OS=Homo sapiens	0.1792453	21.370861
M23090	IGKV3-15*01 OS=Homo sapiens	0.1157895	13.805221
P02649	Apolipoprotein E OS=Homo sapiens	0.0788644	9.4027529
P10909-4	Isoform 4 of Clusterin OS=Homo sapiens	0.0336538	4.012444
Q96KK5	Histone H2A type 1-H OS=Homo sapiens	0.03125	3.7258408
Q16777	Histone H2A type 2-C OS=Homo sapiens	0.0310078	3.6969583
P04004	Vitronectin OS=Homo sapiens	0.0292887	3.4920014
P02768	Serum albumin OS=Homo sapiens	0.0279146	3.3281731
O60814	Histone H2B type 1-K OS=Homo sapiens	0.0238095	2.8387359
P02743	Serum amyloid P-component OS=Homo sapiens	0.0179372	2.1385992
P02760	Protein AMBP OS=Homo sapiens	0.0170455	2.0322768
P02748	Complement component C9 OS=Homo sapiens	0.0143113	1.7062885
P02766	Transthyretin OS=Homo sapiens	0.0136054	1.6221348
P68871	Hemoglobin subunit beta OS=Homo sapiens	0.0136054	1.6221348
P67936	Tropomyosin alpha-4 chain OS=Homo sapiens	0.0120968	1.442261
P60709	Actin, cytoplasmic 1 OS=Homo sapiens	0.0106667	1.2717537
AF449617	IGHG2*04 Homo sapiens	0.0102041	1.2166011
M11737	IGKC*03 Homo sapiens	0.0093458	1.1142702
J00228	IGHG1*01 Homo sapiens	0.0090909	1.083881
P08670	Vimentin OS=Homo sapiens	0.0085837	1.0234069

Figure 4. **The results of laser micro-dissection and liquid chromatography tandem mass spectrometry (LC-MS/MS). The kappa chain constant region (21.37%) and kappa chain III variable region (13.8%) were the predominant components of the nodular lesion depositions (highlighted in yellow). The amyloid P component was 2.14%, which was less than serum albumin (3.33%).**

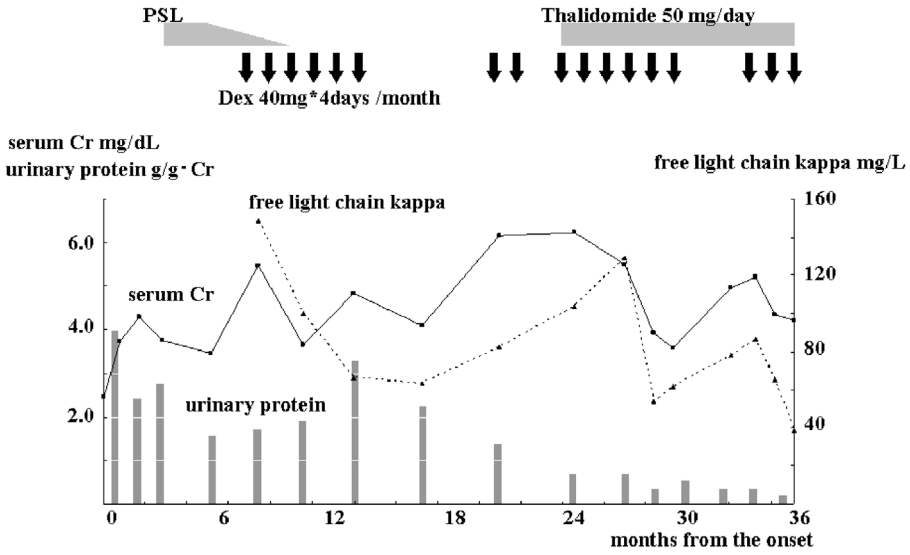

Figure 5. **The clinical course. Dex therapy and Thal-Dex therapy decreased the serum levels of free kappa light chain from 147 to 38 mg/L, and his proteinuria resolved (less than 0.3 g/day). There was no progressive decline in the kidney function, with serum creatinine levels stable around 4.0 mg/dL for 3 years.**

(Fig. 5). Follow-up echocardiography revealed a stable cardiac function, and his NT-proBNP levels had decreased to 557 pg/mL at 18 months after admission.

Discussion

Nodular glomerulosclerosis has been reported to be the most characteristic finding in patients with diabetic nephropathy or amyloid nephropathy. It is also found in patients with dysproteinemias such as LCDD and heavy chain deposition disease (HCDD). Recently, nodular glomerulosclerosis was also observed in fibronectin nephropathy and collagenofibrotic glomerulopathy. In addition to these diseases, in 1989, Herzenberg et al. proposed ING as a new disease entity characterized by the absence of diabetes mellitus, amyloidosis, and immunoglobulin deposition without a known etiology (5). They emphasized the influence of hypertension and smoking. In 2002, Markowirz et al. reported that 23 out

of 5073 kidney biopsy specimens were compatible with ING. There was male predominance (18 males and 5 females), and 96% and 70% of patients had hypertension and nephrotic syndrome, respectively. Serum creatinine levels greater than 1.2 mg/dL were observed in 83% of patients, and 56% of patients developed progressive renal failure over an observation period of 14 months (6). They suggested hypertension and smoking as causes of ING. Nasr and D'Agati also emphasized the role of smoking in ING in 2007 (7).

The present case was initially compatible with ING, because there was no significant deposition of immunoglobulin heavy or light chains, negative Congo red staining of nodular glomerular lesions, normal glucose tolerance, no increase in the number of plasma cells in the bone marrow, no M-proteins in the serum and urine, and a more significant increase in the levels of free light chain kappa than pf lambda. Laser micro-dissection of the nodular lesions and LC-MS/MS revealed that the major deposited proteins consisted of the kappa chain constant region and kappa chain type III variable region, which signifies LCDD (kappa type). We found a similar case in the literature (8), where LC-MS/MS of a nodular pulmonary lesion revealed kappa light chains, signifying LCDD (kappa) of the lung, with negative Congo red staining and an immunofluorescence study.

LC-MS/MS is an extremely useful tool for diagnosing unknown deposition diseases. In amyloidosis, it is known that immunofluorescence and immunohistochemical studies are sometimes negative because the degeneration or decomposition of antigen epitopes occurs during and after amyloid fibril formation. It is reported that mass spectrometry-based proteomics were able to determine the type of amyloidosis in 95% of cases compared with 69% of cases by immunohistochemistry (9). Similarly, 6% of LCDD are reported to show negative findings on immunofluorescence studies (10). Our case revealed the potential value in performing a proteomics analysis, especially when no other approaches reveal the contents of deposition.

The incidence of LCDD has been found to range from 0.33% to 0.5% in kidney biopsy specimens (1-3), which is less than the 1% for amyloidosis (11, 12). Nodular glomerulosclerosis associated with LCDD is emphasized with light microscopy; however, only 60% of LCDD patients have nodular lesions. The remaining patients have mesangial expansion or basement membrane depositions (13). Under electron microscopy, dense granular deposits are present in the mesangial area and subendothelial space without fibrillar structures; however, 8% of patients with LCDD have 8- to 20-nm fibrillar structures present (14). This patient also had random fibrillar structures on electron microscopy. Interestingly, in this case, LC-MS/MS revealed the presence of a serum amyloid P (SAP) component weaker than that of the kappa chain constant region and kappa chain type III variable region and serum albumin. SAP is a normal circulating plasma protein suspected of playing some role in amyloid fibril formation (15). However, the relationship between SAP and LCDD is unclear, and further investigation is necessary. Kappa light chains are predominant in 80-90% of LCDD patients (16, 17), compared to the predominance of lambda chains in amyloidosis. Kappa IV and I light chain variable region LCDD has been reported as the most common type of LCDD (18-20). Kappa III variable region has been also reported; truncated kappa III variable region caused LCDD in one patient (21). When subjected to *in vitro* fibrillar-formation experiments, the kappa III variable type of Bence-Jones protein adopts a fibrillar conformation only at an acidic pH and remains aggregated but not fibrillar at physiological pH (22).

The natural course of LCDD is associated with a very poor prognosis, with 97% of patients having serum creatinine levels greater than 1.2 mg/dL (average, 3.9 mg/dL) at the time of LCDD diagnosis, 39% of patients developing end-stage renal failure over 34 months of observation, and 32% of patients dead at a mean observation duration of 18 months (13). The combination of multiple myeloma (RR 2.75), and extra-renal deposition (RR 2.24) are prognostic risk factors for life (23).

Regarding therapy for LCDD, drugs used to treat multiple myeloma are recommended by the guidelines for multiple myeloma when LCDD patients are complicated with multiple myeloma. In patients with LCDD not accompanied by multiple myeloma, hematopoietic stem cell transplantation and chemotherapy with thalidomide, dexamethasone, bortezomib, lenalinamide, and alkylating drugs are recommended (17). A case report was published of a patient with LCDD that responded to chemotherapy, with no nodular glomerular lesions seven years after chemotherapy (24). It is very important to use adequate drugs for reducing the levels of free light chain.

Regarding the formation of nodular glomerular lesions, dysproteins such as light chain or heavy chain stimulate mesangial cells to produce mesangial matrix components such as collagen and tenascin via NF-kB in LCDD. The proteasome inhibitor bortezomib, which directly interferes with and inhibits NF-kB, is a promising drug for reducing the formation of glomerular nodular lesions (25). We have to monitor serum levels of free light chains and consider chemotherapy to maintain stable levels.

The authors state that they have no Conflict of Interest (COI).

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