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Journal of Clinical Nephrology and Research

Research Article

Kappa Restricted (AL) Amyloidosis Demonstrates Strong PAS and JMS Staining Compared to Lambda Restricted and AA Type Amyloidosis

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Submitted: 10 November 2016

Accepted: 10 January 2017

Published: 12 January 2017

ISSN: 2379-0652

Keywords

- Amyloid
- Light chain restriction
- Kappa light chain
- AL-type amyloidosis
- Amyloid nephropathy

Abstract

Background: We have recently encountered a string of kappa light chain-restricted AL amyloidosis cases in renal biopsies showing an unusual staining profile for amyloid with distinctly strong staining of the amyloid material for periodic acid Schiff (PAS) and Jones Methenamine Silver (JMS); a finding that is opposite the expected results for amyloid.

Study Design: Retrospective cohort study

Setting and Population: Nine patients with biopsy proven renal amyloidosis between January 1, 2009 and December 1, 2015

Factors: Three amyloid subtypes: kappa light-chain restricted AL amyloidosis, lambda light chain-restricted AL amyloidosis and AA type amyloidosis; special stain results

Outcome: Detection of unusual kappa light-chain restricted staining.

Results: Cases with kappa restriction all showed strong staining for PAS and JMS within areas of amyloid, whereas cases with lambda restriction and AA type amyloidosis showed the opposite and expected finding with weak to negative PAS and JMS staining in the area of amyloid deposition.

Limitation: Retrospective study, small sample size

Conclusions: This unusual staining pattern for amyloidosis may be a diagnostic pitfall and lead to the missed diagnosis of amyloid in the setting of kappa light chain-restricted AL amyloidosis.

INTRODUCTION

Amyloidosis is an uncommon systemic disease that results in organ and tissue damage caused by extracellular deposition of abnormal fibrillary proteins that have been associated with at least 25 known types of insoluble proteins [1,2]. Clinical management of these patients is based on the underlying etiology and therefore accurate identification of the type of amyloidosis is a very important component of the diagnostic process [1-3]. The kidney is one of the most frequently involved organs by amyloid deposition. The amyloid may be found within any compartment, but the glomeruli and vessel walls are the most common locations [4-6]. Patients with renal amyloidosis will most often present with sudden or worsening proteinuria that is usually within nephrotic range and can be very high [1].

The two most common types of amyloidosis involving the kidney are light chain restricted amyloid (AL), so-called primary type amyloidosis, often associated with monoclonal gammopathies including MGUS and multiple myeloma, and reactive amyloid A (AA), a so-called secondary type of amyloidosis most often associated with chronic inflammatory diseases such as rheumatoid arthritis, chronic infections, such as osteomyelitis, and certain malignancies [1,2,6,7]. In the United States, the most common type of amyloidosis is AL type, and is most often lambda restricted. However, in developing countries and worldwide, AA amyloidosis is the most common type and is usually associated with chronic infection.

Amyloidosis is routinely diagnosed by histology and immunohistochemistry. By light microscopy, amyloid appears as homogenous pink amorphous extracellular material with hematoxylin and eosin (H&E) stains and usually weak on PAS while negative or weak on JMS. Confirmation by Congo red stain demonstrates orange (or Congophilic) staining of amyloid material and distinctive apple-green brief ringence

when viewed under polarized light (8, 9). Sub typing of the amyloid may be accomplished with immunofluorescence or immunohistochemistry techniques. Immunofluorescence microscopy can demonstrate light chain restriction and confirm the diagnosis of AL type amyloid in most cases. Immunohistochemical stains can be used to highlight specific amyloid proteins such as amyloid A in AA type amyloidosis or transthyretin in some forms of hereditary amyloidosis. In a subset of cases where routine immunofluorescence and immunohistochemical studies cannot definitively type the amyloid material or the Congo red staining is equivocal, further sub typing by laser microdissection and mass spectrometry-based proteomic analysis (LMD/MS) [3,6] is needed. Electron microscopy is routinely performed and shows characteristic thin non-branching and haphazardly arranged fibrils. The light and electron microscopic features of all types of amyloidosis are considered identical and sub typing requires the aforementioned analysis with immunohistochemistry (including immunofluorescence) and/or LMD/MS.

In our institution we have recently discovered cases with kappa light chain-restricted amyloidosis demonstrate a unique staining pattern characterized by strong staining for both PAS and JMS, which is opposite of the expected findings of amyloidosis. In this study, we compared PAS and JMS staining of AL amyloid with kappa restriction to the staining of AL with lambda restriction and AA type amyloidosis. Recognition of this distinctive pattern of staining may prevent a potential diagnostic pitfall and be a useful pre-diagnostic clue for amyloid cases that may be AL type with kappa restriction [8,9].

METHODS

We searched our database for renal biopsies between January 1, 2009 and December 1, 2015 and found 3 cases of kappa light chain-restricted AL amyloidosis, three more cases of lambda light chain-restricted AL amyloidosis and 3 cases of AA type amyloidosis that were collected with the same protocols and transport media. All specimens were fixed in 10% formalin for routine light microscopy, Zeus fixative for immunofluorescence studies and glutaraldehyde for electron microscopy evaluation. All tissue was stained with H&E, PAS, JMS and Masson's trichrome and compared using light microscopy [10,11]. A Congo red stain had been performed at the time of the biopsy and confirmed the diagnosis of amyloid by demonstrating red-orange staining (Congophilia) and apple-green birefringence under polarized light [12].

Cases with light chain restriction were determined by immunofluorescence microscopy using fluoresceinated anti-sera to kappa and lambda immunoglobulin light chains [13]. The cases not demonstrating light chain restriction were evaluated by immunohistochemistry for amyloid A using formalin-fixed, paraffin embedded tissue [12]. Amyloid Amonoclonal antibody binds to serum amyloid A protein deposited within the kidney tissue thus providing qualitative identification of AA type amyloid in these cases. All cases were evaluated using transmission electron microscopy [10]. Tissue blocks were fixed overnight in glutaraldehyde and embedded the next day. Sections were first cut1-micronthick and stained with toluidine blue. Next, they were observed with light microscopy to achieve precise location of key areas for cutting thinner sections at 60 to 90 nm for observation under the electron microscope. The diameter of identified fibrils was measured in nanometers. Only one case showing kappa restriction by immunofluorescence microscopy was sent out for amyloid characterization by mass spectrometry due to the lack of clinical evidence of a paraproteinemia.

This study was approved by the UCLA Institutional Review Board (#15-001766).

RESULTS

The 3 cases with kappa light chain-restricted AL amyloidosis showed strong staining for JMS (Figure 1A) and PAS (Figures 2A) within areas of amyloid deposition, whereas all other cases with lambda light chain-restricted AL and AA type amyloidosis were silver negative (Figures 1B,1C) and weakly-positive on PAS stains (Figures 2B,2C). Masson's Trichrome demonstrated the characteristic blue grey staining (Figures 3A-C) in all cases, while the Congo red showed red-orange staining with apple-green birefringence upon polarization among all cases. Immunofluorescence microscopy confirmed light-chain

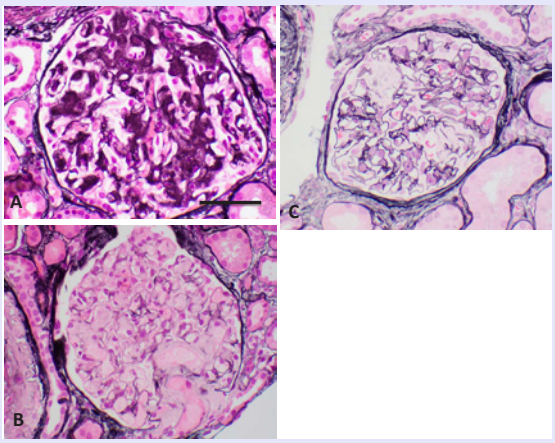

Figure 1 JMS. Magnification 400X. A: AL kappa restricted with strong staining; B: AL lambda restricted, negative, C: AA type, negative. Scale bar 50 microns.

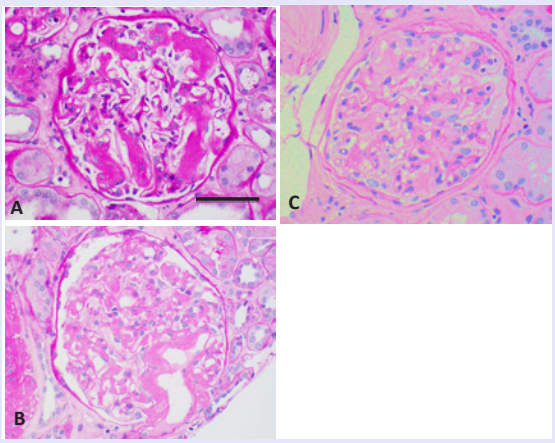

Figure 2 PAS. All magnification 400X. A: AL kappa restricted with strong staining; B: AL lambda restricted, weak staining; C: AA type, weak staining. Scale bar 50 microns.

restrictionwith dominant staining for kappa or lambda within areas of amyloid deposition (Figure 4A-C) with intensities of 4+ each on a 0 to 4 scale. Electron microscopy demonstrated haphazardly arranged fibrils ranging in thickness from 8-12 microns, characteristic of amyloid, and did not show significant ultrastructural differences among the three case types in our study (Figures 5A,5B). There were no pathologic features of diabetic glomerulopathy in any case.

DISCUSSION

In evaluation of amyloidosis on the renal biopsy, the light microscopic findings often show the characteristic pink amorphous material on the H&E stain characteristic of amyloid. However, amorphous extracellular material can easily be mistaken for collagen, but the additional stains in a renal biopsy panel also have characteristic staining qualities (blue-grey on the Masson trichrome stain and negative/weak on the PAS and JMS stains) and the diagnosis can be confirmed by a Congo red stain under polarized microscopy [4,8,9]. The appearance of amyloid by light microscopy may be quite subtle and easily missed if the diagnostic suspicion is not high or if there are staining irregularities. Therefore, the discovery of small foci of amyloid in a renal biopsy sample can be a diagnostic challenge.

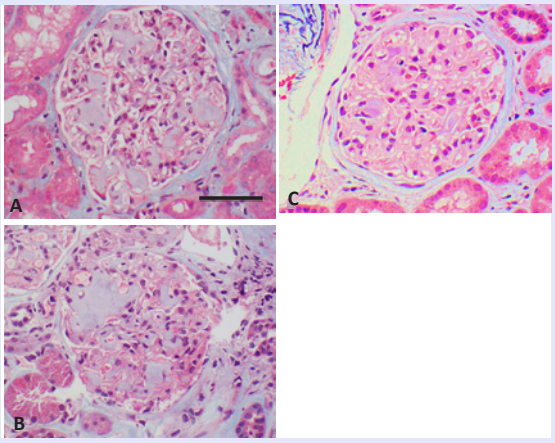

Figure 3 Masson's Trichrome. All magnification 400X. Similar staining for all 3.A: AL kappa restricted; B: AL lambda restricted; C: AA type. Scale bar 50 microns.

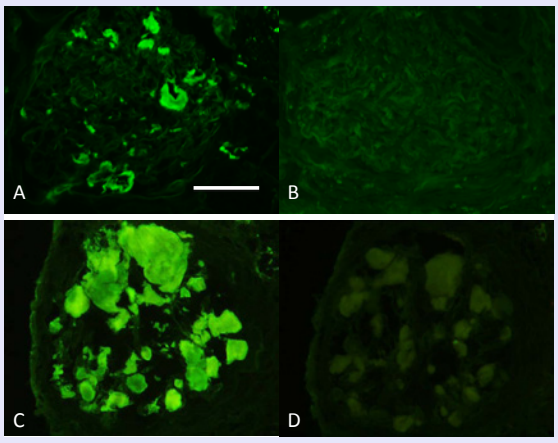

Figure 4 Immunofluorescence. All magnification 400X. A: kappa restriction, 4+B: negative lambda; C: lambda restriction, 4+D: negativekappa. Scale bar 50 microns.

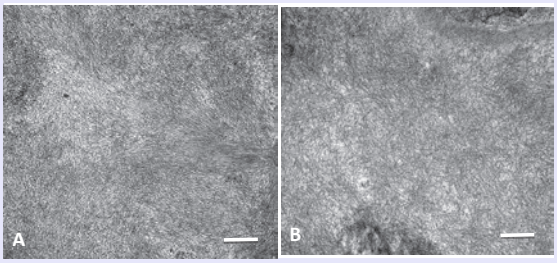

Figure 5 Electron Microscopy. A: Kappa restricted amyloid (Mag. 20,000X). Scale bar 500 nm. B: Lambda restricted amyloid (Mag. 40,000X). Scale bar 250 nm.

The clinical presentation is often nonspecific and a biopsy is required to confirm the diagnosis and exclude other possibilities. In our experience, most centers do not perform a Congo red stain as part of the routine battery of stains in the evaluation of a medical kidney biopsy. Furthermore, renal pathologists may not order a Congo red stain if the PAS and JMS stains have anun characteristic staining pattern for amyloid. In the setting we describe of strongly positive PAS and JMS amyloidosis with Kappa restriction, the diagnosis could easily be missed if the clinical suspicion is low, if the deposits are small or in the setting of a suboptimal biopsy.

The primary consideration in the differential diagnosis of PAS/JMS positive extracellular material is diabetic glomerulosclerosis, a far more common entity than kappa-restricted AL amyloidosis. This fact may result in having a low level of suspicion for amyloid and lead to difficulties in finding the correct diagnosis. Furthermore, the features of amyloid can be subtle by immunofluorescence and electron microscopy studies; glomeruli may be absent in these tissues or EM may not be performed on all biopsies in some centers.

In this comparative study of amyloid types we found that cases of AL amyloidosis with kappa light chain-restriction showed consistent and similarly strong staining for PAS and JMS. All diagnoses were confirmed by Congo red stain and electron microscopy studies. In nearly all cases of amyloidosis of any type the usual findings on both PAS and JMS stains are weak to negative which serves as an important clue against glomerulosclerosis which tends to stain strongly for both stains. Therefore, recognition of this unusual pattern of staining in cases of AL amyloid with kappa restriction may prevent a pathologist from falling into this diagnostic pitfall, particularly if material for immunofluorescence and electron microscopy is unavailable or insufficient. Being aware of this feature of kappa-restricted AL amyloidosis may allow the pathologist a preliminary clue to the correct diagnosis and shorten the time to definitive treatment for the patient.

ACKNOWLEDGEMENTS

Contributions

Research idea and study design: AES, WDW; data acquisition: AES, FHC; data analysis/interpretation: AES, HFC, WDW, MFP-D,

CRL; supervision or mentorship: WDW, MFP-D, CRL. Each author contributed important intellectual content during manuscript drafting and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. AES takes responsibility that this study has been reported honestly, accurately, transparently; that no important aspects of the study have been omitted; and any discrepancies from the study have been explained.

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Cite this article

Sisk AE, Castro FH, Palma-Diaz MF, Lassman CR, Wallace WD (2017) Kappa Restricted (AL) Amyloidosis Demonstrates Strong PAS and JMS Staining Compared to Lambda Restricted and AA Type Amyloidosis. J Clin Nephrol Res 4(1): 1056.