Journal of Nephropathology

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Clinicopathological study of renal amyloidosis and its relationship with renal amyloid prognostic score

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ARTICLE INFO	ABSTRACT
<i>Article type:</i> Original Article	<i>Introduction:</i> The kidney is one of the most common organs involved in systemic amyloidosis. Several studies have attempted scoring kidney amyloid deposits and predicting the outcome. A new
<i>Article history:</i> Received: 9 Jul. 2023 Revised: 17 Oct. 2024 Accepted: 5 Feb. 2025 Published online: 22 Feb. 2025	scoring and grading scheme is proposed, known as renal amyloid prognostic score (RAPS), which provides a better means for scoring renal amyloidosis and predicting renal outcome. <i>Objectives:</i> The present study aims to estimate the clinicopathological and biochemical parameters in renal amyloidosis cases and the role of RAPS in assessing renal outcome and prognosis. <i>Materials and Methods:</i> This retrospective study included all diagnosed cases of renal amyloidosis
<i>Keywords:</i> Amyloidosis, Nephrotic syndrome Apple green birefringence Glomerular filtration rate	from October 2017 to December 2021. Detailed clinical features and laboratory parameters were obtained from medical records, and all renal biopsies were studied using light microscopy (LM) and immunofluorescence. Congo red-stained sections were examined under a polarizer to look for amyloid deposits. RAPS was calculated on a scale of 0 to 31 and was graded from 0 to III. Pearson's correlation coefficient was calculated between RAPS and serum creatinine, as well as between RAPS and estimated glomerular filtration rate (eGFR). <i>Results:</i> Fourteen cases of renal amyloidosis were included, comprising seven cases of primary amyloidosis, of which six showed lambda light chain restriction. RAPS varied from 12 to 27. There was a strong correlation between RAPS and serum creatinine (r= 0.7) and a moderate negative correlation between RAPS and eGFR (r=-0.5). <i>Conclusion:</i> Application of RAPS and, thus, uniform reporting of renal amyloidosis helps assess the disease's severity.

Implication for health policy/practice/research/medical education:

In a retrospective study, we analysed the clinicopathological and biochemical parameters in fourteen patients of renal amyloidosis and assessed whether prognosis can be guided by renal amyloid prognostic score (RAPS). Our results suggest that renal amyloidosis had varied presentation. It adds to the literature that the application of RAPS is a valuable tool and provides an opportunity to have a uniform reporting, therefore the severity and prognosis can be assessed in renal amyloidosis.

Please cite this paper as: Dixit D, Ranade RS, Rani H, Athanikar VS, Revanasiddappa M. Clinicopathological study of renal amyloidosis and its relationship with renal amyloid prognostic score. J Nephropathol. 2025;14(2):e21507. DOI: 10.34172/jnp.2025.21507.

Introduction

Amyloidosis is a term used to describe a group of proteinfolding disorders caused by varied etiological factors but shares some common characteristics. One of the main features of amyloidosis is the deposits' unique staining properties and fibrillar ultrastructural appearance. Interestingly, despite the diversity of these proteins, they all share a beta-pleated sheet secondary structure that results in specific staining patterns and stability under physiological conditions. When viewed under an electron microscope, these proteins appear as non-branching fibrils between 7.5 to 10 nm (1).

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Dixit D et al

The kidney is one of the most common organs to be affected in systemic amyloidosis. Irrespective of the underlying cause, it is also one of the principal causes of mortality. Although the most common presentation is nephrotic syndrome, presentation with renal failure is also known, especially when the vessel wall and medullary zone have significant deposits (2). Several studies have attempted scoring kidney amyloid deposits and predicting the outcome. Sen et al has proposed a scoring and grading scheme called renal amyloid prognostic score (RAPS), which provides a better means for scoring renal amyloidosis and to predict outcomes and compare therapeutic trials (3,4). In this scoring system, six patterns of glomerular involvement by amyloid deposits have been described, similar to the classification of glomerular involvement in systemic lupus erythematosus (3).

Objectives

The present study aims to estimate the clinicopathological and biochemical parameters in renal amyloidosis cases and the role of RAPS in assessing the patient's prognosis.

Materials and Methods

Study design

This was a retrospective study. All the renal biopsy records from October 2017 to December 2021 were reviewed. Cases of renal amyloidosis diagnosed based on light microscopy (LM), examination of Congo red stained slides under polarizer, and direct immunofluorescence (DIF) were considered for the study. Demographic, clinical, and relevant laboratory investigations at the time of renal biopsy were retrieved from medical records.

Two separate cores of renal tissue were analyzed for histological evaluation. LM and DIF studies were included in the analysis. For LM, sections stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), Masson trichome stain (MTS), periodic acid silver methenamine (PSM), and Congo red stain were reviewed. Polarized LM was conducted to examine Congo red-stained slides. Frozen sections were used for the DIF studies. Polyclonal antisera (FITC-conjugated Rabbit Antihuman Antisera manufactured by DAKO from Denmark) against human IgG, IgA, IgM, C3, C1q, kappa, and lambda light chains were used. Immunohistochemistry (kappa and lambda light chains) was performed wherever possible. Inadequate renal biopsies (without single glomerulus with preserved morphology, non-availability of core for immunohistochemistry/DIF) and transplant biopsies were excluded from the study.

Biopsies were classified based on renal amyloidosis prognostic score (RAPS) as per the study conducted by Sen et al (3). The amyloid deposits in biopsies were looked for dominant involvement, i.e., glomerular, interstitial, vascular, or all compartments. The classification of glomerular involvement was classified on a score of 1 to 6 (3). Cumulative RAPS was calculated based on scoring of amyloid involvement of glomerular, interstitial, and vascular compartments; as well as interstitial fibrosis and tubular atrophy, interstitial inflammation, glomerular sclerosis, glomerular class. The renal findings were finally graded according to the RAPS into four grades from 0 to III (Table 1) (3).

Estimated glomerular filtration rate (eGFR) was calculated by the modified MDRD (modification of diet in renal diseases) equation (5). Follow-up details of these patients were obtained until the last visit to the hospital, ranging from three months to three years.

Statistical analysis

The data were entered in excel sheets with an exclusive code assigned to all data. Statistical analysis was carried out using IBM SPSS version 25. Categorical variables were expressed as frequencies and percentages. Continuous variables were expressed as mean if normally distributed and median with an interquartile range for non-normally distributed valuables. Pearson's correlation coefficient was calculated between RAPS and serum creatinine and RAPS and eGFR at the time of presentation. Meanwhile, Pearson's correlation coefficient (r value) of more than \pm 0.5 was considered as strong correlation.

Results

A total of 700 renal biopsies were received during the study period. Of the 700 renal biopsies, 14 cases of renal amyloidosis were diagnosed based on LM aided by Congo red stain. Furthermore, these cases were classified as primary or secondary based on the presence/absence of heavy chain or light chain restriction in DIF. Fourteen cases of renal amyloidosis comprised 2% of all native renal biopsies.

Table 1. Renal amyloid prognostic score (RAPS) and grade (3)

Definition	Grade	RAPS
RAPS		0-31
RAPS = GAP + GA% + VA + IA + Ifib + Iinf + GS		
Grades of renal amyloidosis		
No renal amyloidosis	Grade 0	0
Early renal amyloidosis	Grade I	1–7
Late renal amyloidosis	Grade II	8-15
Advanced renal amyloidosis	Grade III	16 or higher

GA%, percentage of glomerular amyloid deposition; GAP, Class of glomerular amyloid deposition; GS, Glomerular sclerosis; IA, Interstitial amyloid deposition; Ifib, interstitial inflammation and interstitial fibrosis and tubular atrophy; Iinf, Interstitial inflammatory infiltration; VA, Vascular amyloid deposition.

Age ranged from 28 to 72 years, with a mean of 51.5 years. Male: female ratio was 2.5:1. There was an equal number of primary and secondary cases of renal amyloidosis. The most common clinical presentation was nephrotic syndrome (seven cases), and one patient each presented with chronic renal failure, and cerebrovascular accident, since one patient presented with acute kidney injury.

Hemoglobin ranged from 7.6 to 10.7 g/dL, with 60% of cases having anemia at presentation; six patients showed high ESR ranging from 22 to 130 mm at the end of the first hour. Besides, C-reactive protein was raised in three patients, ranging from 8.04 to 16.55 mg/L with a mean of 12.5 mg/L. Additionally, 11 patients showed proteinuria. Nephrotic range proteinuria was reported in six patients with a mean proteinuria of 11.1 g/d.

Bone marrow examination was performed in five cases, out of which one showed features of multiple myeloma and two showed bone marrow plasmacytosis, with one of them showing amyloid deposits in the bone marrow. Electrophoresis was carried out in four cases; one was suggestive of multiple myeloma, and in another one, it was detected as monoclonal gammopathy of undetermined significance. The other two bone marrow biopsies did not show any evidence of plasmacytosis. They were reported as micronormoblastic erythroid hyperplasia and myeloid hyperplasia respectively. In addition, no evidence of monoclonal gammopathy (M protein spike) was seen in the other two cases. Moreover, 10 (71.4%) patients showed high serum creatinine with a mean of 3.25 mg/ dL, while it was within normal limits in the other four

Table 2. Histopathological and biochemical profile of renal amyloidosis patients

(28.6%) cases with a mean of 0.71 mg/dL (Table 2).

Renal biopsy examination revealed the following findings; the average number of glomeruli examined was 15 ranging from 8 to 34. The amyloid deposit was highest in the glomerular compartment (100% of cases), followed by the vascular compartment in 85.7%.

Amyloid deposits appeared pale pink in PAS-stained slides, were Congo red positive, showed apple green birefringence under polarizing microscope, and were silver negative, one case of heavy light chain amyloidosis, where the deposits were PAS and silver positive (Figures 1A to 1F).

Crescents were seen in only one case (7.1%) (Figure 2). Immunofluorescence was performed in all the cases. Six of the 14 cases showed lambda light chain restriction, and one showed heavy chain (IgG) and light chain (lambda) restriction and were classified as cases of primary amyloidosis. The other seven cases did not show any light chain restriction and were classified as secondary amyloidosis cases (Figures 3A to 3D). The etiology of secondary amyloidosis was tuberculosis in one of the cases and was unknown in others.

Extrarenal amyloid deposits were observed in two cases. One case showed deposits in bone marrow with reactive plasmacytosis. The other showed cardiac amyloidosis and was a case of IgG-lambda-associated primary amyloidosis.

RAP Score ranged from 12 to 27, and RAPS grade in seven cases (50%) were of grade II. The rest of the cases were of RAPS grade III. Pearson's correlation coefficient between RAPS and serum creatinine was r=0.7, and between RAPS and eGFR was r=0.5, indicating a strong

Age (y)	Gender	GAP	GA	VA	IA	IFib	IInf	GS	RAPS	RAPS grade	SCr (mg/dL)	eGFR (mL/min/1.73m ²)
60	М	3	3	2	0	1	1	2	12	II	0.86	96.4
76	М	2	3	3	1	1	3	3	16	III	4.53	13.6
49	F	4	4	3	0	0	0	1	12	II	0.61	110.8
43	М	4	5	2	0	0	1	0	12	II	1.98	39.4
55	М	2	3	4	1	2	1	1	14	II	1.04	78.8
60	М	2	2	1	0	3	3	2	17	III	1.1	72.6
55	F	4	5	2	3	1	1	1	22	III	1.11	54.2
40	М	4	5	2	2	4	4	1	22	III	4.2	16.8
70	F	6	4	4	2	4	3	4	27	III	12.1	3.3
35	F	3	3	3	1	4	3	2	19	III	1.88	32.4
28	М	3	3	1	0	1	2	2	12	II	0.6	160
50	М	3	3	2	0	2	2	2	14	II	2.1	34
72	М	3	3	0	2	1	1	3	13	II	2.5	25.5
28	М	3	5	0	2	3	3	2	18	III	0.79	117

M, Male; F, Female; GAP, Class of glomerular amyloid deposition; GA, Percentage of glomerular amyloid deposition; VA, Vascular amyloid deposition; IA, Interstitial amyloid deposition; Ifib, Interstitial inflammation and interstitial fibrosis and tubular atrophy; Iinf, Interstitial inflammatory infiltration; GS, Glomerular sclerosis; RAPS, Renal amyloid prognostic score; SCr, Serum creatinine.

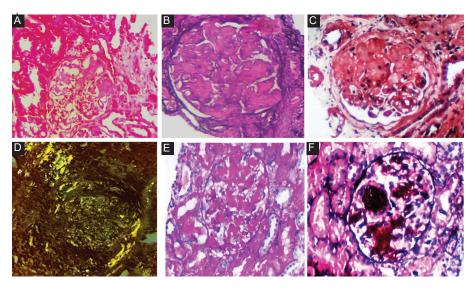


Figure 1. (A) Extracellular amorphous eosinophilic deposits depicting amyloid in mesangium and obliterating capillary lumen (H&E, 40×). (B) Amyloid deposits negatively stained with Gomori's methenamine silver (GMS, 40×). (C) Brick red positivity of amyloid (Congo red, 40×). (D) Amyloid deposits in glomeruli and interstitium displaying apple green birefringence under polarizing microscopy (Congo red, 40×). (E) A case of heavy-light chain amyloidosis displaying PAS positive amorphous extracellular deposits in glomeruli (PAS, 40×). (F) Silver positive deposits in mesangial region in a case of heavy-light chain amyloidosis (GMS, 40×)

positive correlation between RAPS and serum creatinine across with a strong negative correlation between RAPS and eGFR.

Patients were followed up from three months to three years' duration. Three years follow-up was seen in a patient with RAPS 12, while the patient with RAPS 22, as a case of IgG-lambda-associated primary amyloidosis, died due to cardiac amyloidosis.

Discussion

Amyloid deposits can involve many organs in the body, but renal involvement is the most common clinical presentation as nephrotic syndrome and progressive renal failure (6,7). It is a significant cause of morbidity in these patients and usually progresses to end-stage renal disease in a significant number of patients without treatment. Amyloidosis is classified based on the

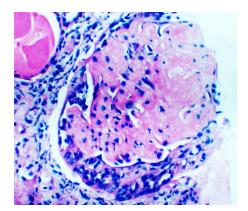


Figure 2. Extracellular amorphous eosinophilic deposits depicting amyloid in mesangium and obliterating capillary lumen and a circumferential cellular crescent (PAS, 40×)

precursor protein that forms the amyloid fibrils and as either systemic or localized based on the distribution of amyloid deposits. Immunoglobulin (Ig) light chain (AL), Ig heavy chain (AH), amyloid A (AA), the familial or hereditary amyloidosis (TTR, fibrinogen A, lysozyme, apolipoprotein AI [apoAI], apoAII, gelsolin, and cystatin), senile systemic amyloidosis, and beta 2-microglobulin amyloidosis are the major types of systemic amyloidosis. In AL amyloidosis, an Ig light chain or light chain fragment produced by clonal plasma cells deposits in the tissue as

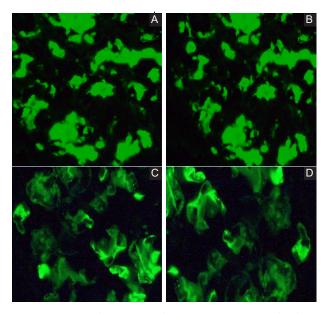


Figure 3. (A) Smudgy IgG (3+) deposits in mesangium under direct immunofluorescence. (B) Smudgy lambda light chain deposits (3+) in mesangium under direct immunofluorescence. (C) Negative for lambda light chain deposits under direct immunofluorescence. (D) Negative for kappa light chain deposits under direct immunofluorescence.

amyloid. Any organ except the central nervous system can be a site of AL amyloid deposition. In nearly 50% to 80% of individuals, the kidneys are affected (8-12). Since the kidney is frequently involved in various types of systemic amyloidosis, renal biopsy is often the method to diagnose the disease. Diagnosis of renal amyloidosis is based on histologic demonstration of amyloid deposits by Congo red stain showing an orange-red appearance under LM and apple green birefringence under polarizer. Technically, Congo red staining can be challenging, especially when the tissue sections are 5 microns thick. It can even be falsely positive if tissue is over-stained (8). Amyloid deposits in the kidney can be found in all the compartments, i.e., glomerular, vascular, and interstitial, but usually, glomerular lesions predominate (3,4).

The age of presentation in various studies ranged from 35 to 63 years which was 28 to 76 years in the present study. Various studies have shown male preponderance similar to our study (13-17). Nephrotic syndrome is the most common presentation in the present study. Kalle et al reported nephrotic syndrome in 47.5% with mean proteinuria of 4.16 g/d in cases with primary amyloidosis and 5.46 g/d in cases with secondary amyloidosis, respectively (4). High serum creatinine was recorded in most cases in various studies (4,13-17).

In the present study, there was an equal number of primary and secondary amyloidosis cases comparable to the study carried out by Kalle et al. However, in the study conducted by Sen et al, there were significantly more cases of secondary amyloidosis (3,4). Crescents were seen in one case (7.1%) of secondary amyloidosis, which was a known case of pulmonary tuberculosis with RAPS 22. Concomitant glomerular crescents have been reported with renal amyloidosis (3,18). Likewise, study carried out by Verine et al has reported crescents in 17.6% of the cases of secondary renal amyloidosis (19).

In the present study, cases of primary amyloidosis showed lambda light chain restriction in 86% of the cases, while one showed heavy light chain (IgG-lambda) restriction. Kalle et al, has reported that 92% of the primary amyloidosis cases show lambda light chain restriction (4). The case with heavy light chain restriction showed silver-positive amyloid deposits. Cases with heavy-light chain restriction usually show negative silver deposits. However, an atypical staining pattern, such as silver-positive deposits, has been described in some of these cases (20).

In the present study, seven cases did not show any light chain restriction and were classified as secondary amyloidosis cases. However, the cause of secondary amyloid could not be elicited in them except for a known case of pulmonary tuberculosis. Sen S et al have reported familial Mediterranean fever as the most common etiology of AA type and multiple myeloma as the most common etiology in non-AA type renal amyloidosis (3). Meanwhile, Verine et al has reported chronic infection followed by chronic inflammation as the most common cause of secondary amyloidosis (19).

RAPS in the present study reported an equal number of grades II and III. Similar findings have been reported by Sen et al, while grade I lesion were more than grade III in a study performed by Kalle et al (Table 3) (3,4).

Extrarenal amyloid deposits were observed in two cases, one showing deposits in bone marrow, with reactive plasmacytosis in the bone marrow. Cardiac involvement was seen in one patient with IgG-lambda-associated primary amyloidosis. This patient clinically presented with arrhythmia and macroglossia and later died. Priyamvada, et al have also reported a case of IgG-lambda-associated primary amyloidosis presenting with complete heart block (20). Different grades of RAPS in our study were compared with RAPS grades noted in other studies too (Table 3).

Serum creatinine and eGFR are widely used to assess renal function. In the present study, we observed a strong positive correlation between RAPS and serum creatinine and a moderate negative correlation between RAPS and eGFR. Hence, applying RAPS in biopsy-proven cases of renal amyloidosis might dictate patient prognosis.

Conclusion

The kidney is one of the most common organs involved in systemic amyloidosis, with nephrotic syndrome being the most common presentation. A multidisciplinary approach, including clinical, biochemical, and renal biopsy findings, including Congo red stained slides and DIF, helps to diagnose the disease and assess the severity. The application of RAPS is a valuable tool and provides uniform reporting to assess the severity of renal amyloidosis and patient prognosis.

Table 3. Comparison	of RAPS in	present study	with other studies
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RAPS grade	Present study (n=14)	Kalle et al (n=40) ⁴	Sen et al (n=305) ³
0	-	-	-
Ι	-	45%	13.1%
II	50%	52.5%	53.1%
III	50%	2.5%	30.8%
Inadequate for scoring	-	-	3%

Limitations of the study

A small sample size limited the present study. Etiologic factors of secondary amyloidosis were unknown in the majority of the cases. Effects of confounding variables were not considered, and further investigations like electron microscopy and mass spectrometry were not performed to determine the subtype of amyloid deposits.

Acknowledgments

We acknowledge the histopathology lab technical staff of SDM University for their support and help.

Authors' contribution

Conceptualization: Deepti Dixit, Ranjana Shashidhar Ranade, Hephzibah Rani.

Data Curation: Deepti Dixit.

Formal analysis: Ranjana Shashidhar Ranade, Manjunath Revanasiddappa.

Investigation: Ranjana Shashidhar Ranade, Manjunath Revanasiddappa, Deepti Dixit.

Methodology: Ranjana Shashidhar Ranade, Deepti Dixit, Vidisha Sharatchandra Athanikar, Hephzibah Rani.

Project administration: Ranjana Shashidhar Ranade, Vidisha Sharatchandra Athanikar.

Resources: Deepti Dixit, Ranjana Shashidhar Ranade.

Supervision: Ranjana Shashidhar Ranade, Manjunath Revanasiddappa.

Validation: Ranjana Shashidhar Ranade, Hephzibah Rani.

Visualization: Ranjana Shashidhar Ranade, Manjunath Revanasiddappa.

Writing-original draft: Deepti Dixit, Hephzibah Rani.

Writing-review and editing: Ranjana Shashidhar Ranade, Vidisha Sharatchandra Athanikar, Manjunath Revanasiddappa.

Conflicts of interest

The authors declare that they have no competing interests.

Data availability statement

The data of the patients in the current study are available from the corresponding author on reasonable request.

Ethical issues

This retrospective study review was conducted in accordance with the World Medical Association Declaration of Helsinki. The institutional ethical committee at SDM University approved all the study protocols (SDMCDS IEC.No. 2021/Medical/Pathology/S/15). Accordingly, written informed consent was taken from all participants before any intervention. Besides, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

None.

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