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CLINICAL CORRELATION OF HISTOPATHOLOGICAL CLASSIFICATION, SCORING, AND GRADING IN AMYLOID NEPHROPATHIES: SINGLE-CENTER EXPERIENCE

Büşra Yaprak Bayrak[1*], Çiğdem Vural[1], Kerem Teke[2] Metin Ergül[3] Necmi Eren[3]

[1]Kocaeli University, Faculty Of Medicine, Department of Medical Pathology, Kocaeli, Turkey; [2]Kocaeli University, Faculty Of Medicine, Department of Urology, Kocaeli, Turkey; [3]Kocaeli University, Faculty Of Medicine, Department of Nephrology, Kocaeli, Turkey.

ORCID iD: Büşra Yaprak Bayrak: 0000-0002-0537-3127; Çiğdem Vural: 0000-0002-9405-9112; Kerem Teke: 0000-0001-9030-4662; Metin Ergül: 0000-0002-0706-6550; Necmi Eren: 0000-0002-2708-3470

***Sorumlu Yazar / Corresponding Author:** Büşra Yaprak Bayrak, **e-posta / e-mail:** bsr2004_86@hotmail.com

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Abstract

Objective: Amyloidosis is disorder of various etiologies in which abnormally folded fibrillary protein deposits with more than thirty forms infiltrate into extracellular spaces of affected organs. Renal involvement is clinically characterized by decreased estimated glomerular filtration rate (eGFR) and proteinuria. The aim of present study was to classify and grade renal amyloidosis cases using renal amyloid prognostic score (RAPS) systems, correlate clinical data and chronic kidney disease (CKD) stages.

Methods: We retrospectively analyzed kidney biopsies of 45 patients diagnosed with renal amyloidosis applied between 2017-2022 to our department and scored each of patients according to RAPS.

Results: 8.9% of patients had RAPS score 1, 53.3% had 2 and 37.8% had 3. Urea, serum creatinine and proteinuria levels of RAPS3 patients were significantly higher and eGFR levels were lower compared to RAPS1 patients ($p<0.01$). According to CKD stages, no significant difference was observed in glomerular amyloid deposition class and score, vascular and interstitial amyloid deposition scores, and glomerular sclerosis ($p>0.05$). The interstitial fibrosis, inflammation values and RAPS scores were found to be significantly higher in advanced CKD stages ($p<0.05$). Majority of patients at CKD stage 1-2 had RAPS score 2 (73.68%), while 57.1% of at stage 3 and 66.7% at stage 4-5 had RAPS score of 3 ($p=0.0015$).

Conclusion: As a result, the intestinal fibrosis, inflammation values, RAPS scores were significantly higher in advanced CKD stages. Distribution pattern of amyloid in the renal parenchyma compartment, grade of RAPS and eGFR were associated with urea/creatinine, proteinuria levels and thus with CKD stage.

Keywords: *Grading, amyloid nephropathy, scoring, classification.*

Introduction

Amyloidosis encompasses a group of diseases characterized by the accumulation of an abnormally folded protein of more than thirty forms with an insoluble fibrillar structure.[1,2] The most prevalent types of amyloidosis found in plasma cell dyscrasias and chronic inflammatory diseases are light chain amyloidosis (AL) and amyloid A amyloidosis (AA), respectively. Other examples include the long-term hemodialysis, amyloid transthyretin in senile systemic amyloidosis, and beta 2 microglobulin in hereditary polyneuropathies.[1,3] The majority of hereditary amyloidosis cases are linked to inherited inflammatory conditions such familial Mediterranean fever (FMF). The morbidity of amyloidosis is influenced by the organs implicated, their level of functional compromise, and the amyloid fibrils' direct toxicity.[4]

Amyloidosis is also divided into localized and systemic types depending on the level of participation. Systemic amyloidosis frequently affects the kidney, which has a significant negative impact on individuals with chronic renal disease's prognosis (CKD). The range of renal morphological changes is extremely diverse, and to some extent, the chemical structure dictates the major site of involvement.[5] The most typical location for early fibril deposition is the glomerulus.[6] Morbidity is greatly impacted by additional related abnormalities like glomerular sclerosis, tubular atrophy, interstitial inflammation, and fibrosis. The nephrotic syndrome is the most typical manifestation of renal amyloidosis. Nonetheless, individuals with renal failure may manifest if the deposits are mostly medullary or vascular. It can result in renal disease in final stages if neglected.[7] According to a recent study, the dialysis reliance after the diagnosis and median patient survival for AL were 36.3 months and 50 months, respectively, those were 52.9 months and 18 months for AA.[7]

AA and AL types of renal amyloidosis can be defined immunohistochemistry (IHC) and immunofluorescence (IF) examinations.[8] IF is used to show the clonality of light chains for primary amyloidosis and IHC is used to determine the acute phase reactants secondary amyloidosis, typically serum amyloid A (SAA). The clinical course of amyloidosis can be predicted as well as the diagnosis can be made using renal biopsy. Many studies have attempted to score the renal amyloid deposits.[1,3,4]

Sen et al. scored renal amyloidosis by grading system which offers a superior tool for comparing and predicting the therapeutic outcomes.[4] It is essential to score amyloid deposits using the standardized criteria in order to evaluate the disease progression and to promote uniformity and inter-institutional comparison of reports. A grading system for renal amyloidosis called Renal Amyloid Prognostic Score (RAPS) is based on the morphology, location, pattern, and severity of amyloid deposition.[1,4,8] The type of renal amyloidosis also showed geographical differences. While the Western world shows the predominance of the primary or light chain forms, the secondary type is common in developing countries, including our country.[4] Therefore, in this retrospective study, we aimed to classify and grade the patients with renal amyloidosis using RAPS scoring systems, and then correlate the clinical data with CKD stages.

Methods

Study Cohort

This retrospective one-center study recruited 45 patients with CKD stages 1–5 (including dialysis patients) selected from CKD patients who are admitted at our department between 2017 – 2022 and diagnosed with renal amyloidosis histopathologically. Acute illnesses that required hospitalization within the last three months, glomerulonephritis without renal amyloidosis, and patients who refused to join the cohort were all eliminated from the study. The Declaration of Helsinki's guiding principles were followed in every step of this study involving human subjects. All individuals who took part in the study provided written informed consent. The regional ethics committee approved the protocol of this study (Date: 2021/11/14, Decision No: GOKAEK-2022/18.39 Project No: 2022/311).

Data Collection

Clinical examination of CKD patients has been described in detail before.[9] All sociodemographic and medical history data, clinical features and laboratory findings were collected from the hospital records. The medical history included the onset, etiology, co-morbidities, and known vascular risk factors, such as diabetes, arterial hypertension, dyslipidemia, bone marrow involvement, functional liver abnormalities, vascular diseases (coronary heart disease, stroke/transient ischemic attack, peripheral artery disease), treatment of CKD, and current medications.

CKD Diagnosis

According to the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation, CKD was defined as the presence of chronic proteinuria or a reduced estimated glomerular filtration rate (eGFR) of 90 mL/min per 1.73 m2 in two measurements collected at three months apart.[10] There are six categories for eGFR. A longer period more than 3 months with an eGFR below 60 mL/min per 1.73 m^2 indicated an impaired renal function, while longer periods with decreasing eGFR values indicated more the severity of kidney damage. Patients at CKD stage 1-2, who were at an early-onset illness, have eGFR levels that are normal to mildly reduced (60 to 90 mL/min per 1.73 m^2). Patients at CKD stage 3a-3b have modest to moderately reduced eGFR (respectively, 45–59 mL/min per 1.73 m^2). eGFR levels which reduced significantly (15-29 at stage 4 to 15 mL/min per 1.73 m^2 at stage 5) are a sign of severe illness and renal failure at stage 4-5.[11]

Laboratory Measurements

Total lab data were collected from all patients. The measurements from the blood included levels of β2-microglobulin (RR: 0.60-2.28 mg/dL), total bilirubin (RR: 0.3-1.0 mg/dL), direct bilirubin (RR: 0-0.4 mg/dL), alkaline phosphatase (ALP) (RR: 40-130 IU/ml), aspartate aminotransferase (AST) (RR: 15-20 IU/L), alanine aminotransferase (ALT) (RR: 10-40 U/L), gama glutamyl transferase (GGT) (RR: 0-65 U/L), lactate dehydrogenase (LDH) (RR: 90-240 U/L), total cholesterol (RR: <200 mg/dL), LDL cholesterol (RR: <100 mg/dL), triglycerides (RR: <150 mg/dL), ejection fraction value (RR: 50-70%), plasma NT pro-BNP (RR: 10-30 pg/mL), troponin-I (RR: <0.06 ng/mL), blood urea (Reference range (RR): 16.6-48.5

mg/dl), eGFR (RR: 80-140 mL/min/1.73m2), serum creatinine (CR) (RR: 0.7-1.2 mg/dl), serum albumin (RR: 39.7-49.4 d/dL), proteinuria (mg/24h), serum kappa (RR: 3.3-19.4 mg/L), serum lambda (RR: 5.71-26.3 mg/L), serum kappa/lambda ratio (RR: 0.26 – 1.65).

Microscopic Examination

Hematoxylin and eosin (H&E), Masson trichrome, periodic acid-Schiff, methenamine silver-periodic acid-Schiff, and Congo red were used for light microscopic examination (Figure 1, 2a). Amyloid was detected on H&E stained samples of all patients and observed as amorphous, eosinophilic and congophilic extracellular material and as apple green birefringence when viewed through a polarizer light.

Fluorescein isothiocyanate (FITC)-conjugated antibodies against kappa and lambda light chains with IgM, IgA, IgG, C3 and C1q at a dilution of 1:30 were used for direct immunofluorescence on fresh frozen kidney biopsy, followed by a 30-minute incubation period. The slides were examined under the filtered Olympus BX50 Phase Contrast Fluorescent Microscope.

Immunohistochemical Examination

On a fully automated immunostainer, IHC was carried out using ready-to-use antibodies against AA (Dako, Germany), as well as kappa and lambda light chains. The secondary antibody, goat anti-rabbit anti-mouse immunoglobulin, was labeled using polyhorseradish peroxidase polymer, and 3.3'-diaminobenzidine was utilized as the chromogen (Figure 2b).

In each case, IF and IHC immunostaining results for kappa, lambda, and SAA were analyzed in comparison to the positive and negative controls. Positive controls included the tissues from the cases documented, while negative controls included the tissues without antibody addition. The interpretation of light chains was performed by mesangial staining.

Histopathological Evaluation of Renal Amyloidosis

The overall dominant involvement of amyloid deposits including glomerular, interstitial, vascular involvement were examined in the biopsies. Glomerular participation was rated from 1 to 6 using the scoring and grading system of Sen et al.[1] The glomerular class, interstitial fibrosis and tubular atrophy, interstitial inflammation, and glomerular sclerosis were all considered in the amyloid scoring in addition to the degree of their participation. The total score was identified as RAPS. Following classification of each cases, a RAPS grade was determined ranged from 0 to 3 (Table 1), and then compared based on the stages of CKD.

AA amyloidosis was assessed by a history of chronic infection, no evidence of light chain limitation, and positive IHC expression of AA. AL amyloidosis was identified using immunoelectrophoresis and immunofixation to show the presence of bone marrow plasma cells and light chain restriction.

Follow-up

Of the total cohort of 45 patients, two patients died during two-year follow-up due to renal involvement. None of the patients underwent kidney transplantation. Seven patients who had renal dysfunction for ≥3 months and eGFR ≤ 10 ml/min/1.73 m^2 underwent hemodialysis. One patient died due to an uremic complication.

Statistical Analysis

All statistical analysis was performed by GraphPad Instat package program. The Kolmogorov-Smirnov test was used to determine how the variables were distributed. ANOVA and the Tukey-Kramer Multiple Comparisons Test were used to assess normally distributed continuous variables. The Kruskal-Wallis and Dunn's Multiple Comparisons Test was used to examine continuous variables having non-normally distributions. For categorical variables, the Chi-squared Test for Independence was applied. Pairwise comparisons were made using the Mann-Whitney test. p values of *0.05*, *0.01* and *0.001* were regarded as significant.

Results

77.8% of all patients were male, mean age was 54.8 ± 14.9 years (Table 2). The most common comorbidities in all patients were hypertension (33.3%) and malignancy (26.6%). 20% of the patients were diagnosed with AL and 80% of them were diagnosed as serum AA. 28.9% of the patients were diagnosed at CKD stage 1, 13.3% at stage 2, 31.1% at stage 3, 11.1% at stage 4 and 15.6% at stage 4. Liver and cardiac function tests are presented in Table 2.

8.9% of the patients had a RAPS score of 1 (n=4), 53.3% had score of 2 (n=24), and 37.8% had score of 3 (n=17) (Table 3). The mean age of patients with RAPS 2 and 3 scores was significantly higher than that of RAPS1 patients (*p=0.0007*). Gender distribution was comparable between three RAPS groups (*p=0.432*). The most common comorbidities were hypertension and malignancy among patients with RAPS 2 and 3 scores, and the difference between RAPS groups was at borderline significance (*p=0.064*). The AL and AA distributions of the patients did not differ between the RAPS groups (*p=0.565*) (Table 2). Total cholesterol, LDL cholesterol, triglyceride, serum albumin levels and kappa/lambda ratio among renal findings were not significant between the RAPS groups (*p>0.05*). Urea, serum creatinine and proteinuria levels of RAPS3 patients were significantly higher and eGFR levels were lower compared to those of RAPS1 patients (*p<0.01*). While all RAPS1 patients were in CKD stages 1 and 2, the majority of RAPS3 patients (47.1%) were in CKD stage 3 (*p=0.0207*) (Table 3).

When the histopathological findings of the patients were compared according to CKD stages, no significant difference was observed in terms of glomerular amyloid deposition class and score, vascular and interstitial amyloid deposition scores, and glomerular sclerosis (*p>0.05*) (Table 4). On the other hand, interstitial fibrosis, inflammation values and RAPS scores were found to be significantly higher in advanced stages (*p<0.05*). The majority of patients (42.1%) with CKD stage 1-2 had an interstitial fibrosis score of 1. The majority of those with stage 3 (42.9%) had a score of 3, and the majority of those with stage 4-5 (41.7%) had a score of 4 (*p=0.0227*). The interstitial inflammation score was 1 in the majority (52.7%) of patients with CKD stage 1-2. 42.9% of patients with CKD stage 3 had an inflammation score of 2 or 3. 41.7% of the patients with CKD stage 4-5 had an inflammation score of 3 and 33.3% had an inflammation score of 4 (*p=0.0009*). While the majority of patients (73.68%) at CKD stage 1-2 had a RAPS score of 2, 57.1% of those at stage 3 and 66.7% of those at stage 4-5 had a RAPS score of 3 (*p=0.0015*) (Table 4).

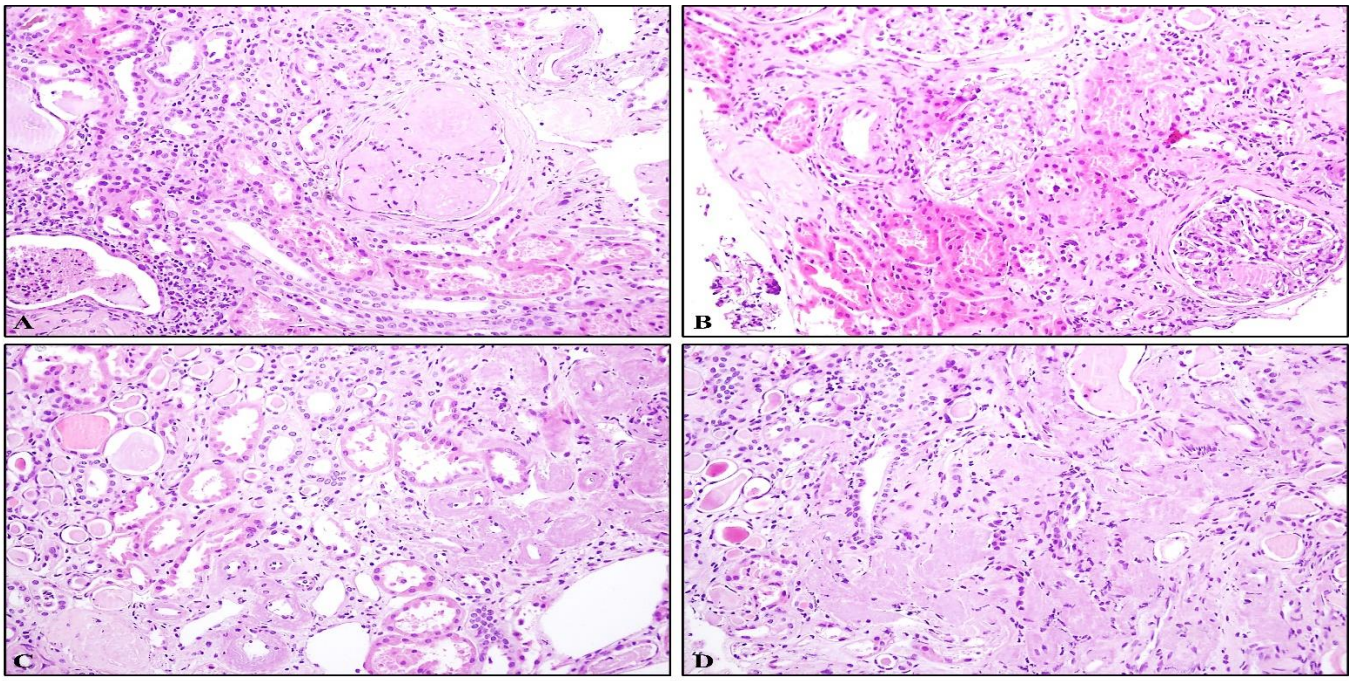

Fig 1. A. Histology of renal amyloidosis. Extensive deletion of glomerular architecture by amorphous amyloid (H&E, x200). **B.** Amyloid deposition in less than 50% of the glomerulus (H&E, x200). **C.** Tubular and perivascular amyloid deposition (H&E, x200). **D.** Interstitial amyloid material (H&E, x200).

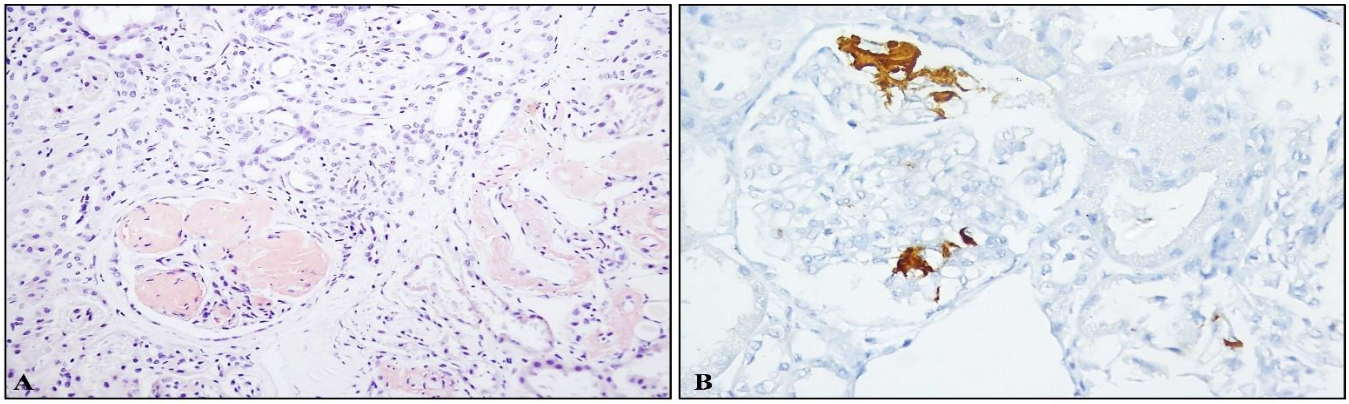

Fig 2. A. Amyloid staining positively with Congo red in the glomeruli and vessel walls (x200). **B.** Amyloid deposition in a portion of the glomerulus that stains positively with Amyloid AA (x400).

Table 1. Scoring of histopathological findings in renal amyloidosis, numerical codes

Type of renal amyloidosis	Histopathological findings	Score
Glomerular amyloid deposition classification (GAP)	Absent, hilar, minimal mesangial, focal mesangial, mesangial capillary, membranous, global sclerotic	0-6
Percentage of glomerular amyloid deposition (GA %)	0.1%–10%, 11%–25%, 26%–50%, 51%–75%, 76%–100%	0-5
Vascular amyloid deposition (VA)	Absent, minimal, focal, moderate, severe amyloid deposition	0-4
Interstitial amyloid deposition (IA)	Absent, minimal, focal, moderate, severe amyloid deposition	0-4
Interstitial fibrosis and tubular atrophy (Ifib)	0.1%–10%, 11%–25%, 26%–50%, 51%–75%, 76%–100%	0-4
Interstitial inflammatory infiltration (Iibf)	0.1%–10%, 11%–25%, 26%–50%, 51%–75%, 76%–100%	0-4
Glomerular sclerosis (GS)	0.1%–10%, 11%–25%, 26%–50%, 51%–75%, 76%–100%	0-4

Table 2. Demographic data and non-renal laboratory findings of patients diagnosed with renal amyloidosis

	Total (N=45)
Age (year), Mean± SD	54.8 ± 14.9
Gender, N (%)	
Male	35 (77.8)
Female	10 (22.2)
Additional illness, N (%)	
FMF	4 (8.9)
AS	4 (8.9)
RA	1 (2.2)
HT	15 (33.3)
DM	2 (4.4)
Malignancy	12 (26.6)
Other	7 (15.6)
Amyloid type, N (%)	
AL	9 (20)
AA	36 (80)
CKD stage, N (%)	
1	13 (28.9)
2	6 (13.3)
3	14 (31.1)
4	5 (11.1)
5	7 (15.6)
Liver Function Tests, Median [Min-Max]	
β2-microglobulin	4.4 [1.1 – 31.1] mg/dL
Total bilirubin	0.24 [0.03 – 0.91] mg/dL
Direct bilirubin	0.065 [0 – 0.3] mg/dL
ALP	84 [7.2 – 379] IU/ml
AST	18.8 [10.7 – 51] IU/L
ALT	12.2 [2.6 – 30.6] U/L
GGT	32 [6 – 507] U/L
LDH	199.5 [114 – 546] U/L
Cardiac function test, Median [Min-Max]	
Ejection fraction value	65% [55 – 80]
Plasma NT pro-BNP	1060 [85 – 32700] pg/mL
Troponin-I	26 [10 – 220] ng/mL

ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AS, Ankylosing spondylitis; AST, Aspartate aminotransferase; CKD, Chronic kidney disease; DM, Diabetes mellitus; FMF, Familial Mediterranean fever; GGT, Gama glutamyl transferase; HT, Hypertension; LDH, Lactate dehydrogenase; RA, Rheumatoid arthritis.

Table 3. Comparison of demographic data and renal laboratory findings of patients diagnosed with renal amyloidosis according to renal amyloid prognostic score (RAPS)

	RAPS 1 (N=4)	**RAPS 2 (N=24)**	**RAPS 3 (N=17)**	***p value***
Age (year), Mean± SD	30.5 ± 7.3	55.1 ± 13.5**	60.3 ± 12.6***	***0.0007***
Gender, N (%)				
Male	4 (100)	19 (79.2)	12 (70.6)	*0.432*
Female	0 (0)	5 (20.8)	5 (29.4)	
Additional diseases, N (%)				
FMF	2 (50)	2 (8.3)	0 (0)	*0.064*
AS	1 (25)	3 (12.5)	0 (0)	
RA	0 (0)	1 (4.2)	0 (0)	
HT	0 (0)	6 (25)	9 (52.9)	
DM	0 (0)	2 (8.3)	0 (0)	
Malignancy	0 (0)	7 (29.2)	5 (29.4)	
Other	1 (25)	3 (12.5)	3 (17.6)	
Amyloid type, N (%)				
AL	0 (0)	5 (20.8)	4 (23.5)	*0.565*
AA	4 (100)	19 (79.2)	13 (76.5)	
Total Cholesterol (mg/dl), Median [Min-Max]	271.6 [207-394]	300 [131.8-522]	287.2 [122-673.8]	*0.413*
LDL Cholesterol (mg/dl), Median [Min-Max]	211.6 [132-327.5]	205 [73.7-434.9]	200 [68-455.3]	*0.385*
Triglyceride (mg/dl), Median [Min-Max]	199.9 [159.3-250]	197.9 [80-449]	176.6 [73-986.7]	*0.694*
Urea (mg/dl, Median [Min-Max]	25.8 [17.2-41.8]	41.3 [21-137.3]	76.4 [21.8-208.0]**,††	***0.0001***
Serum CR (mg/dl), Median [Min-Max]	0.78 [0.58-0.97]	1.19 [0.16-7.97]	2.33 [0.68-10.98]**,††	***0.0006***
Serum ALB (d/dl), Median [Min-Max]	27.8 [10.8-31.7]	16.8 [1.2-34.1]	18.2 [2.1-39.6]	*0.204*
Proteinuria (mg/24 h), Median [Min-Max]	1240.9 [37.1-3067.3]	10513 [366.9-41998]**	8478.4 [2622.9-15155]*	***0.0047***
Kappa/lambda ratio, Median [Min-Max]	-	0.775 [0.3 – 3.49]	0.35 [0.26 – 2.28]	*0.629*
eGFR (mL/min/1.73m2), Median [Min-Max]	105.1 [81.7-135.9]	75.7 [4.9-177.2]	32.4 [3.9-109]**,††	***0.0003***
CKD stage, N (%)				
1	3 (75)	9 (37.5)	1 (5.9)	***0.0207***
2	1 (25)	5 (20.8)	0 (0)	
3	0 (0)	6 (25)	8 (47.1)	
4	0 (0)	2 (8.3)	3 (17.6)	
5	0 (0)	2 (8.3)	5 (29.4)	

ALB, Albumin; AS, Ankylosing spondylitis; CKD, chronic kidney disease; CR, Creatinine; DM, Diabetes mellitus; FMF, Familial Mediterranean fever; HT, Hypertension; RA, Rheumatoid arthritis.
*$p<0.05$, **$p<0.01$, ***$p<0.001$ vs RAPS1 group. ††$p<0.01$ vs RAPS2 group.

Table 4. Comparison of histopathological findings of patients with renal amyloidosis according to chronic kidney disease stage

	Total (N=45) N (%)	**Stage 1 ve 2 (N=19)** N (%)	**Stage 3 (N=14)** N (%)	**Stage 4 ve 5 (N=12)** N (%)	***p* value**
GAP class.					
1	1 (2.2)	1 (5.3)	0 (0)	0 (0)	
2	2 (4.4)	0 (0)	1 (7.1)	1 (8.3)	
3	3 (6.7)	3 (15.8)	0 (0)	0 (0)	*0.231*
4	17 (37.8)	8 (42.1)	4 (28.6)	5 (41.7)	
5	2 (4.4)	2 (10.5)	0 (0)	0 (0)	
6	20 (44.4)	5 (26.3)	9 (64.3)	6 (50)	
GA score					
1	1 (2.2)	1 (5.3)	0 (0)	0 (0)	
2	2 (4.4)	0 (0)	1 (7.1)	1 (8.3)	
3	3 (6.7)	3 (15.8)	0 (0)	0 (0)	*0.364*
4	16 (35.6)	8 (42.1)	4 (28.6)	4 (33.3)	
5	23 (51.1)	7 (36.8)	9 (64.3)	7 (58.4)	
VA score					
0	6 (13.3)	5 (26.3)	0 (0)	1 (8.3)	
1	6 (13.3)	3 (15.8)	1 (7.1)	2 (16.7)	
2	15 (33.3)	5 (26.3)	7 (50)	3 (25)	*0.345*
3	6 (13.3)	3 (15.8)	2 (14.3)	1 (8.3)	
4	12 (26.7)	3 (15.8)	4 (28.6)	5 (41.7)	
IA score					
0	6 (13.3)	5 (26.3)	0 (0)	1 (8.3)	
1	23 (51.1)	11 (57.8)	5 (35.7)	**7** (58.4)	
2	6 (13.3)	1 (5.3)	4 (28.6)	1 (8.3)	*0.148*
3	5 (11.1)	1 (5.3)	2 (14.3)	2 (16.7)	
4	5 (11.1)	1 (5.3)	3 (21.4)	1 (8.3)	
Ifib score					
0	2 (4.4)	2 (10.5)	0 (0)	0 (0)	
1	13 (28.9)	8 (42.1)	3 (21.4)	2 (16.7)	
2	12 (26.7)	6 (31.6)	4 (28.6)	2 (16.7)	***0.0227***
3	12 (26.7)	3 (15.8)	6 (42.9)	3 (25)	
4	6 (13.3)	0 (0)	1 (7.1)	5 (41.7)	
Iinf score					
0	3 (6.7)	2 (10.5)	1 (7.1)	0 (0)	
1	14 (31.1)	10 (52.7)	1 (7.1)	3 (25)	
2	11 (24.4)	5 (26.3)	6 (42.9)	0 (0)	***0.0009***
3	13 (28.9)	2 (10.5)	6 (42.9)	5 (41.7)	
4	4 (8.9)	0 (0)	0 (0)	4 (33.3)	
GS score					
0	15 (33.3)	8 (42.1)	4 (28.57)	3 (25)	
1	5 (11.1)	4 (21.05)	1 (7.1)	0 (0)	
2	5 (11.1)	3 (15.8)	1 (7.1)	1 (8.3)	*0.050*
3	8 (17.8)	4 (21.05)	3 (21.4)	1 (8.3)	
4	12 (26.7)	0 (0)	5 (35.7)	7 (58.4)	
RAPS score					
1	4 (8.9)	4 (21.05)	0 (0)	0 (0)	
2	24 (53.3)	14 (73.68)	6 (42.9)	4 (33.3)	
3	17 (37.8)	1 (5.3)	8 (57.1)	8 (66.7)	***0.0015***

IA, Interstitial amyloid deposition; Ifib: Interstitial fibrosis; Iinf, Interstitial inflammation; GA, Glomerular amyloid deposition; GAP class., Glomerular amyloid deposition classification; GS, Glomerular sclerosis; RAPS, Renal amyloid prognostic score; VA, Vascular amyloid deposition.

Discussion

In this retrospective study, we classified 45 patients diagnosed with renal amyloidosis using the RAPS scoring system and compared their demographic, clinical and laboratory data based on CKD stages. We determined that the mean age of patients with high RAPS scores was higher, and the urea, serum creatinine and proteinuria values associated with renal function tests were significantly higher and eGFR rates were lower, especially in patients with RAPS score 3. Then, we grouped the cases according to CKD stages and correlated them with their histopathological findings, and as a result, we found that the intestinal fibrosis, inflammation values and RAPS scores were significantly higher in advanced stages.

CKD, hypertension and rheumatoid arthritis are among the most common underlying diseases of renal amyloidosis.[2] Almost half of the individuals with renal amyloidosis in a case series done in US were reported to have hypertension.[12] In our study, the hypertension and malignities showed the higher frequency among the comorbidities of CKD patients with renal amyloidosis. FMF is another predisposing inflammatory disease for amyloidosis, which had a low prevalence in our study. However, in Turkey, the hereditary periodic fevers and infections are responsible for a larger proportion of cases of AA amyloid.[4]

Renal biopsy is collected from cases with clinically suspected renal involvement. The overall renal biopsy incidence of amyloidosis ranges from 1.3 to 4%.[4] The diagnosis is confirmed by the accumulation of acellular, eosinophilic material in the glomeruli, tubulo-interstitial and vessel walls in the kidney parenchyma.[1,4] IF, histochemistry and IHC findings may be helpful in determining the amyloid type. The degree and localization of the accumulation affects the clinical course of disease. For this reason, a scoring is usually done when an amyloid deposition is observed in pathological samples.[4] Various histological grading scores have been proposed for renal amyloidosis. The altered form of scoring system suggested by Sen et al. is the most used one which is based on the glomerular injury pattern.[1] But their system is not relevant to the clinical data. This was developed in a cohort where 90% of patients had renal AA.[5] Systems developed in the AL cohort by Rubinstein et al. and Hoelbeek et al. included the glomerular, interstitial, and vascular deposits which were predictive for end-stage renal disease.[6,7] In our study, we determined RAPS scores in CKD patients using the scoring system developed by Sen et al. and found that 8.9% of patients had a RAPS score of 1, 53.3% had RAPS score of 2, and 37.8% had RAPS score of 3, and patients with high RAPS scores were in the more advanced CKD stage.

Tsai et al. reported that 80% of patients with AL and non-AL amyloidosis had nephrotic syndrome (mean daily proteinuria 6.9±4.73 g) and 40% had low GFR (<50 ml/min).[13] In another series from Spain, it was reported that 69.5% of the amyloidosis cases collected over a 15-year period had nephrotic syndrome (mean proteinuria 6 g/day), and GFR was below 60 ml/min in 50% of AL group and 70% of AA group.[14] In a retrospective series of 373 patients by Bergesio et al., 35.3% of AL patients and 43% of AA patients had chronic renal failure at stage 5 (dialysis).[15] The mean proteinuria was 4.9 g/day in AL patients and 5 g/day in AA patients. In a study by Ayar et al. GFR level was found below 60 ml/min in 95.2% of patients with AL amyloidosis and 56.8% of patients with AA amyloidosis, and the mean proteinuria was 4.24 g/day.[16] We determined that the patients with high RAPS scores among all AL and AA patients had high proteinuria levels and low eGFR rates, and therefore, high RAPS scores could be associated with advanced CKD.

Clinically, AA most frequently emerge by the signs and symptoms of kidney involvement.[4] At the time of diagnosis, a proteinuria and/or renal impairment is present in almost all cases. The advanced age, high SAA level, hypoalbuminemia, and end-stage renal disease has been correlated with an increased rate of mortality.[17] The distribution of AA amyloidosis varies around the world according to the geographical region[18], and it is very common in the European and developing countries.[19] FMF has been identified as the primary etiology of AA renal amyloidosis in the epidemiological research from Turkey. Male gender and 40–50 years of age are the predominant demographics affected by this condition.[20] In a total (AL+AA) cohort by Hoelbeek et al., the significant clinicopathological correlations were detected at diagnosis and an incidence rate of renal amyloidosis significantly differed between age groups and peaked in the 65–79 years age. In addition, the males were more frequently affected than females in Netherlands.[7] In our study, AA accounts for 80% of 45 CKD patients and the mean age of patients with higher RAPS score was significantly higher than the patients with lower RAPS score, suggesting that the older age may be correlated with the renal amyloidosis progression among CKD patients.

AL amyloidosis ultimately leads to destruction of tissues and progressive disease. The kidney is the most commonly affected organ in AL amyloidosis. A study revealed that the proteinuria was the most common renal manifestation, and 70% of the patients had a nephrotic syndrome.[21] For the identification of amyloid deposits in renal tissues, a histologic confirmation based on Congo red is necessary. Findings from light microscopy alone are insufficient to define the class of amyloid protein. In the process of amyloid subtyping, the clinical history as well as direct IF on frozen tissue or by IHC on fixed samples are crucial.[22] The systemic forms of amyloidosis with renal involvement, such as AA amyloidosis, amyloidosis with heavy chain deposition, fibrinogen Aa, or ALECT2 (leukocyte chemotactic factor 2) deposition, must also be differentiated from AL amyloidosis.[23] In our study, direct IF and fibrinogen, Kappa or lambda positivity were found in all of our patients diagnosed with AL amyloidosis. In others, positivity was obtained with IHC and Amyloid AA in which paraffin sections were made. The presence of ALECT2 deposits could not be demonstrated in our cases since the immunostaining for these deposits is not very common in our clinical practice.

In a study by Kalle et al. involved 40 cases of biopsy-proven renal amyloidosis, 90% of the renal involvement was glomerular, either alone or in combination with other compartments.[22] In the lack of glomerular deposition, there was no sign of purely vascular or interstitial involvement. There were no changes in the distribution of amyloid in the various kidney compartments among the 12 primary and 23 secondary amyloidosis cases.[22] The arterial and arteriolar deposits (56%), interstitial deposits (58%), glomerular deposits (97%), and tubular basement membrane deposits (8%) were noted in a large case series of 407 renal amyloidosis patients.[23] In the present study, we compared the amyloid depositions in glomeruli, vascular compartments, interstitial region and tubular basement membrane according to the CKD stages. Similar to the

literature, in the present study, more amyloid formation was observed in the glomerular compartment compared to other renal compartments (tubule, interstitium, vessels). Only the scores of fibrosis and inflammation in the interstitial compartment did significantly differ among the CKD stages, resulting in a significant difference in total RAPS scores.

There are limitations of this study. The retrospective nature of study was an important limitation, since some hospital records of patients were undescriptive or incomplete. Other important limitation is the small sample size. Another limitation was related to the IHC study at our center, which was only performed for AA cases but not to identify the other subtypes of amyloidosis. Moreover, no electron microscopic examination was performed for renal biopsies. We included all AL and AA cases in the study, but we did not included other type of amyloidosis, suggesting that the correlations may overestimate the true association between CKD stage and RAPS scores, since all of these correlations were only significant in total cohort and the sample size of patients with AL was limited.

Conclusion

As a result, the intestinal fibrosis, inflammation values, RAPS scores were significantly higher in advanced CKD stages. Distribution pattern of amyloid in the renal parenchyma compartment and grade of RAPS and also eGFR levels are associated with the urea/creatinine, proteinuria levels and thus with CKD stage. In order to evaluate the prognostic importance of histopathological classification, scoring and grading in amyloid nephropathies, further studies are needed to include large numbers of patients.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Compliance with Ethics Statement

Ethical consent was obtained from the local ethics committee for this study. The study protocol was approved by the Local Ethical Committee of Non-invasive Clinical Research (Date: 2021/11/14, Decision No: GOKAEK-2022/18.39 Project No: 2022/311).

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Authors' contributions

Study idea/Hypothesis: BYB, CV; Data preparation: BYB, CV, ME; Analysis: BYB, ME; Literature review: BYB, CV, KT; Manuscript writing: BYB; Critical Review: BYB, CV, KT, ME, NE.

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