

# A 16-year Survey of Clinicopathological Findings, Electron Microscopy, and Classification of Renal Amyloidosis

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## What's Known

- Electron microscopy plays an important role in the diagnosis of various renal diseases such as amyloidosis.
- The main pathological classifications of renal amyloidosis are based on glomerular involvement and the number of involved main renal components (glomerulus, tubule, vessels, and interstitium). However, a comparison between these classifications has not been made.

## What's New

- Light microscopy did not lead to a definite diagnosis in approximately half of the patients and one-third tested negative with Congo red staining. Electron microscopy investigation prevents misdiagnosis.
- Classification based on glomerulus involvement had a higher correlation with clinical features and is a better predictor of the clinical manifestations.

## Abstract

**Background:** Electron microscopy (EM) is a valuable tool in the diagnosis of renal amyloidosis, particularly in the early stages of the disease. In Iran, studies on EM and the clinical features of renal amyloidosis are scarce. The objective of the present study was to survey EM investigations, pathological classifications, and clinical features of renal amyloidosis.

**Methods:** This cross-sectional study was performed in Shiraz, Iran, during 2001-2016. Out of 2,770 kidney biopsies, 27 cases with a diagnosis of renal amyloidosis were investigated. EM investigation and six staining procedures for light microscopy (LM) were performed. Two pathological classifications based on glomerular, peritubular, perivascular, and interstitial involvement were made. Finally, the association between these classifications and the clinical features was assessed. Chi-square, Fisher's exact, Independent *t* test, and Multiple logistic regression analysis were used. P values < 0.05 were considered statistically significant.

**Results:** In 51.9% of the cases, the clinical diagnosis was nephrotic syndrome. Proteinuria and edema were the most prevalent clinical manifestations. The role of EM investigation for diagnosis was graded "necessary" or "supportive" in 48.2% of the patients. In the classification based on glomerular classes, variables such as the mean blood pressure (P=0.003), history of hypertension (P=0.02), creatinine > 1.5 (P=0.03), and severe tubular atrophy (P=0.03) were significantly higher in class B (advanced amyloid depositions).

**Conclusion:** EM plays an important role in the diagnosis of renal amyloidosis. EM in conjunction with LM investigation with Congo red staining is recommended, to prevent misdiagnosis of patients with a clinical suspicion of renal amyloidosis. Among different pathological features of renal amyloidosis, the severity of glomerular amyloid depositions had a clear relationship with clinical presentations.

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**Keywords** • Amyloidosis • Microscopy • Electrons • Proteinuria • Nephrotic syndrome • Kidney

## Introduction

Amyloidosis encompasses a group of disorders characterized by deposition of insoluble fibrils, predominantly in the extracellular space of the tissues.<sup>1</sup> Although amyloid fibrils can deposit in

any organ,<sup>2</sup> the kidney is the most common site in systemic amyloidosis.<sup>3</sup> Irrespective of other underlying causes, kidney involvement is the main cause of mortality.<sup>4</sup> Clinical findings of renal involvement are proteinuria, nephrotic syndrome, and renal failure.<sup>5, 6</sup> Direct toxicity of the amyloid fibrils deposition and, as a result, a decrease in renal function leads to increased mortality.<sup>7</sup> Besides, pathological findings such as interstitial fibrosis and tubular atrophy significantly contribute to morbidity.<sup>8, 9</sup>

The diagnosis of renal amyloidosis is established during routine processing of kidney biopsy, using light microscopy (LM) and electron microscopy (EM) investigations, by detecting amyloid deposits in each of the pertinent histological compartments of the kidney. Staining with Congo red is one of the most widely used procedures to identify amyloid deposits.<sup>10</sup> EM is useful in the diagnosis of amyloidosis, particularly in the early amyloid deposition, by confirming the presence of amyloid fibrils. In some cases, amyloidosis might be undetectable with LM since fibril deposition is minimal, and staining with Congo red could be negative.<sup>9</sup> Due to non-specific clinical symptoms of renal amyloidosis in the early stages of the disease, clinical diagnosis is easily missed, which leads to delayed management and a poor clinical outcome. This can also occur in other diseases that are in the early stages and only detectable by EM investigation.<sup>11</sup> Some studies have suggested a scoring scheme for evaluating renal amyloid deposition.<sup>12, 13</sup> In this regard, the scoring and grading system proposed by Sen and colleagues seems to be the most comprehensive system.<sup>9</sup>

Several studies have been conducted on different clinical and pathological aspects of renal amyloidosis.<sup>3, 8</sup> However, to the best of our knowledge, EM studies on renal amyloidosis in Iran are scarce and have only covered a short time frame. Thus, the objective of the present study was to survey EM investigations, pathological findings, and clinical features of renal amyloidosis over 16 years. Besides, the relative frequency of diagnosed renal amyloidosis using EM was investigated, and a comparison between the two modified classifications was made.

## Materials and Methods

In a cross-sectional study, all the 2,770 kidney biopsies of patients referred to our EM unit during 2001-2016 were investigated. The EM unit at Nemazee Hospital, affiliated to Shiraz University of Medical Sciences (Shiraz, Iran), is one of the oldest and the main medical center

in southern Iran. All the patients diagnosed with renal amyloidosis by EM investigation were included in the study. Samples from kidney transplant patients and those that did not contain kidney tissue and glomeruli (due to poor kidney biopsy technique) were excluded. Eventually, the biopsies of 27 patients (0.97% of all kidney biopsies) were used in this study.

Written informed consent had been obtained prior to renal biopsies. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (code: IR.sums.med.rec.1397.208).

### *Light Microscopy and Transmission Electron Microscopy*

The renal biopsy tissues were cut into sections, and processed for LM and EM investigations. In the case of LM, 3-5  $\mu$ m serial sections were fixed in formalin, embedded in paraffin, and then stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Jones methenamine silver, Masson trichrome, and Congo red. All of the materials were purchased from Sigma-Aldrich, Germany.

Based on the estimated amount of renal cortex involvement, interstitial fibrosis and tubular atrophy were graded on a semiquantitative scale.<sup>14</sup> The tubulointerstitial damage was categorized as mild (<25%) and moderate to severe ( $\geq$ 25%). In addition, a specimen was cut into small blocks, and routinely processed for EM investigation. A set of 1  $\mu$ m sections was taken and stained with toluidine blue (Sigma-Aldrich, Germany) to detect glomeruli under the light microscope (Olympus BX41, Japan). Then, grids were analyzed under the *Transmission Electron Microscopy* (TEM; Leo 906, Germany). For each case, the data were analyzed using LM and the clinical. Then, the laboratory findings were analyzed using EM to characterize ultrastructural changes of the tissues.

### *Diagnosis of Renal Amyloidosis*

The final diagnosis of renal amyloidosis was based on the presence of non-branching randomly oriented fibrils in different parts of the renal cortex specimen that lacked hollow center and measured 8-12 nm in thickness in EM at  $\times$ 80,000 magnification. In all cases, an LM investigation was performed and visualized as extracellular amorphous eosinophilic material on H&E. For the detection of amyloid with Congo red staining, the presence of salmon-colored deposits was assessed under polarized light, which showed an apple-green birefringence.

### *Grades and Classes of Amyloid Deposition*

All biopsies were evaluated with EM for

amyloid deposition in one of the pertinent histological compartments of the kidney, including the glomerular (G), peritubular (T), perivascular (V), and interstitial (I) areas. For detailed grading of the kidney involvement, we developed a modified version of the scoring system proposed by Sen and Sarcik.<sup>9</sup> Four grades of kidney involvement by amyloid deposition were defined according to a semi-quantitative scale. In this grading system, the extent of amyloid deposits in the glomerular, peritubular, interstitial, and perivascular areas was characterized as either a negative or positive deposit (table 1). Considering the importance of the renal function of glomerular deposition, and in order to obtain a better impression of it, we developed a modified version of the classification proposed by Sen and Sarcik.<sup>9</sup> This classification is based on the percentage of glomerular involvement (table 1). In addition, for better analyses, particularly multiple logistic regression, we defined two main grades (A and B) based on the grading system (table 1). Grade A (non-severe) included grades 1, 2, and 3, and grade B (severe) included grade 4. Similarly, glomerular deposition classes 0 to 4 were classified as class A (non-severe) and class 5 was included in class B (severe) (table 1). All specimens were appraised and scored by an expert renal pathologist. Then, all the plastic sections were stained with toluidine blue, which included both the medulla and cortex of the kidney. These were re-reviewed to check the reliability of the scoring system.

#### *The Role of EM in Diagnosing Amyloidosis*

Before EM, an LM investigation was performed in all studied cases. For each patient, the primary LM diagnosis was evaluated and compared. The contribution of EM to the diagnosis of amyloidosis was assessed and graded as necessary, supportive, and non-helpful.

- Necessary: If the final LM and EM diagnoses were incompatible.
- Supportive: If the LM study only suggests

(not certainly) renal amyloidosis, and EM study confirms the definite diagnosis of renal amyloidosis.

- Non-helpful: If the EM diagnosis was fully consistent with the LM diagnosis, and EM would not have been required to confirm the diagnosis.

#### *Clinical and Laboratory Data*

The available hospital records and pathology requisition forms (including clinical and laboratory data) of all patients were reviewed. Medical records of the patients were retrieved, and the clinical data were extracted.

#### *Statistical Analysis*

Statistical analysis was performed using SPSS software, version 19 (IBM, USA). Quantitative and qualitative variables were expressed as mean±SD and frequency (%), respectively. The independent *t* test, Chi-square test, and Fisher's exact test were used as appropriate. Multiple logistic regression analysis was used to assess the odds of each characteristic on the class or grade of amyloidosis. P values < 0.05 were considered statistically significant.

## Results

Of the 2,770 renal biopsies, 27 (0.97%) cases were diagnosed as renal amyloidosis, of which 16 (59.3%) were male and 11 (40.7%) were female patients. The mean age of the patients was 55.9±2.3 years (range: 34-80 years). The majority of the patients (81.5%) were admitted to Nemazee Hospital, affiliated to Shiraz University of Medical Sciences (Shiraz, Iran). Clinical diagnosis, clinical manifestation, underlying diseases, and drug consumption history of the patients are illustrated in figure 1.

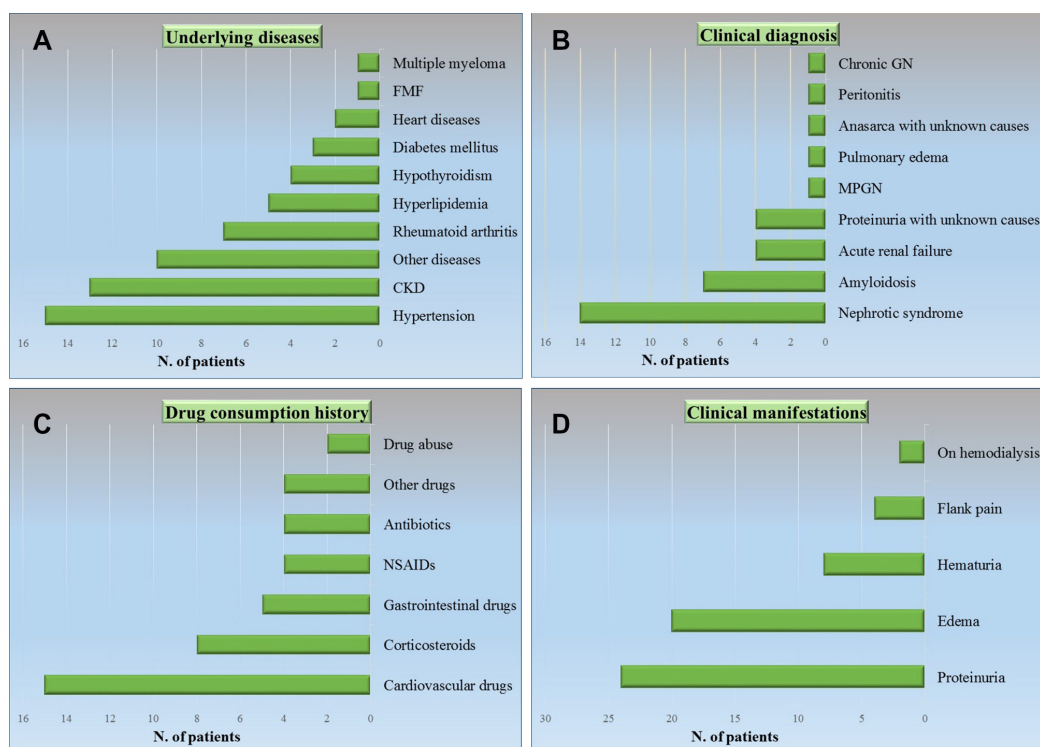
#### *Histopathological and Electron Microscopic Findings*

Mean glomerulus numbers of the EM and LM investigations were 6.3 and 14.4, respectively. The mean percentage of the cortex in the sample

**Table 1:** Amyloid deposition grading system and glomerular deposition classes in 27 patients with renal amyloidosis

Glomerular deposition classes		Number of cases	Grading system		Number of cases		
Class A	Class 0	No amyloid deposition	2	Grade A	Grade1	G	1
	Class 1	Minimal mesangial (<10%)	2		Grade2	(G+V) or (G+I)	16
	Class 2	Mild mesangial (10-25%)	2	Grade3	G+V+T	1	
	Class 3	Focal mesangiocapillary (26-50%)	2	Grade B	Grade4	G+V+T+I	9
	Class 4	Diffuse mesangiocapillary (51-75%)	7				
Class B	Class 5	Advanced (>76%)	14				

G: Glomerular amyloid deposition, T: peritubular amyloid deposition, V: Perivascular amyloid deposition, I: Interstitial amyloid deposition



**Figure 1:** A) Underlying diseases, B) clinical diagnosis, C) drug consumption history, and D) clinical manifestations of patients with renal amyloidosis from 2001 to 2016. FMF: Familial Mediterranean fever, CKD: Chronic kidney disease, GN: Glomerulonephritis, MPGN: Membranoproliferative glomerulonephritis, NSAID: Nonsteroidal anti-inflammatory drug

**Table 2:** Misdiagnosis or concurrent pathology in light microscopy study

LM misdiagnosis or concurrent pathology	Number of cases
Diffuse global glomerulosclerosis	3
Focal segmental glomerulosclerosis	2
Membranoproliferative glomerulonephritis type II	1
Mesangioproliferative glomerulonephritis	1
Poststreptococcal glomerulonephritis	1
Benign nephrosclerosis	1

LM: Light microscopy

tissue was 68% in LM. Positive Congo red staining (range: strong to weak) was observed in 66.7% of the patients. Crescents were not observed in any of the cases. EM investigation for the diagnosis of 4 (14.8%) cases was categorized as “necessary”, 9 (33.3%) as “supportive”, and 11 (40.7%) as “non-helpful” groups. The number of misdiagnosed or concurrent pathology cases in the LM investigation is presented in table 2. In addition, in 7 (25.9%) of the cases, EM investigation was recommended in the LM report for better diagnosis.

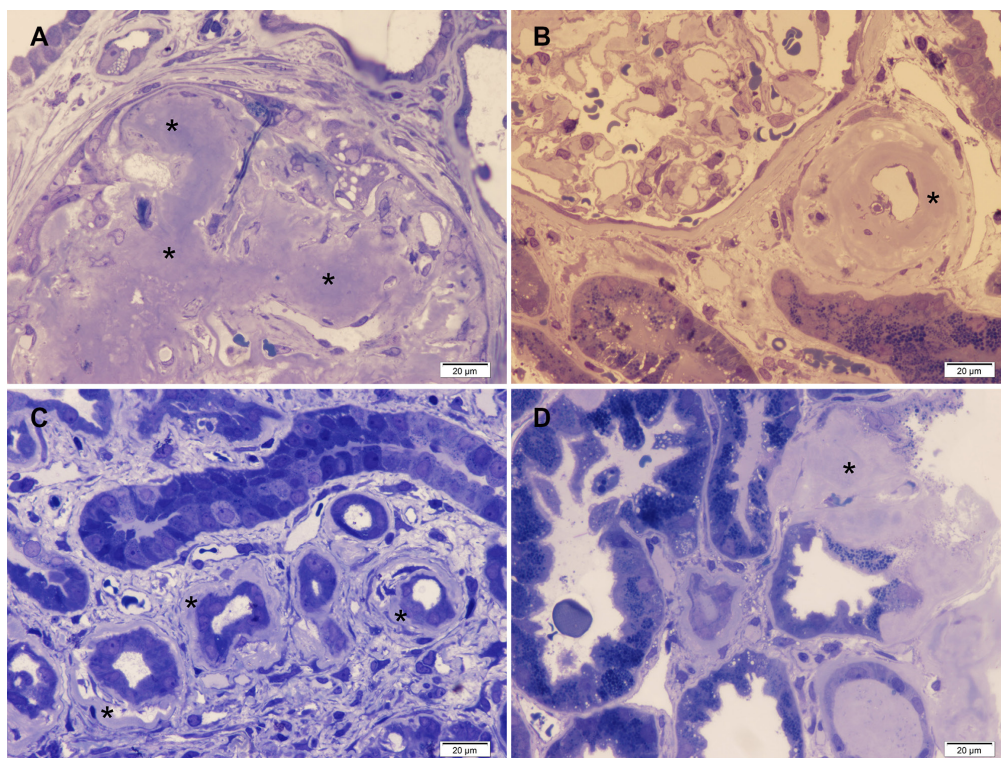
Plastic sections from resin blocks stained with toluidine blue helped to determine amyloid deposition in various parts of the kidney. In terms of amyloid deposition site, all cases had various degrees of glomerular involvement; classified into six classes and two main groups (table 1 and figure 2A). Extraglomerular involvement was observed in 26 cases (predominantly peritubular

in 10, perivascular in 25, and the interstitial area in 11 cases), which were categorized into four grades and two main groups for statistical analysis (table 1; figures 2B, 2C, and 2D).

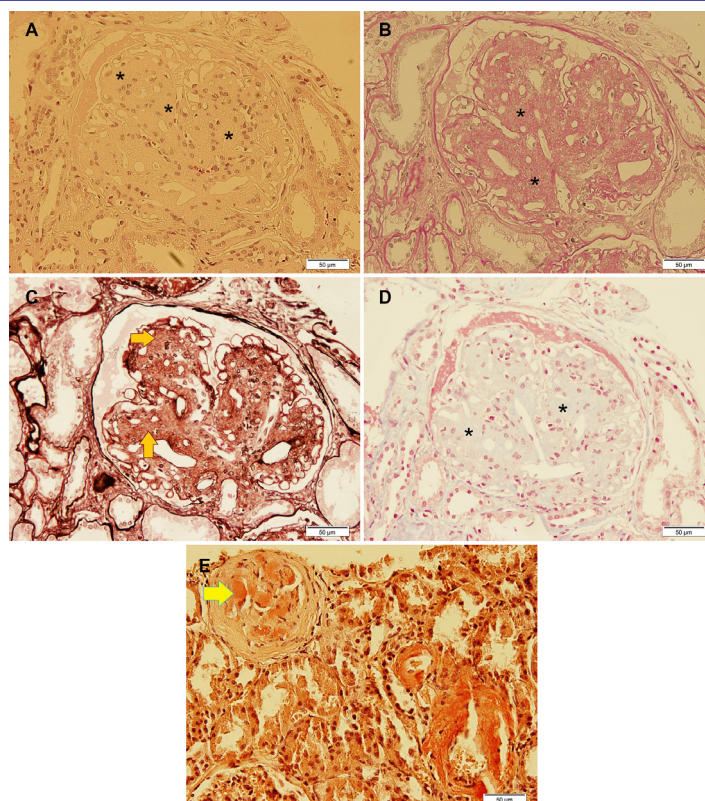
The mean±SD of tubular atrophy and interstitial fibrosis in the EM and LM investigations were 16.3±16.1, 7.6±1.9 and 20.2±18.5, 18.3±17.7, respectively. The LM findings of renal biopsies with five different staining procedures are presented in figure 3. The EM investigation of renal biopsy samples from all the patients revealed the presence of randomly oriented un-branching fibrils (mean diameter: 8-12 nm), involving the glomerular mesangial, peritubular, perivascular, and interstitial areas (figure 4).

#### Correlation between Pathological Classifications and Clinical Features

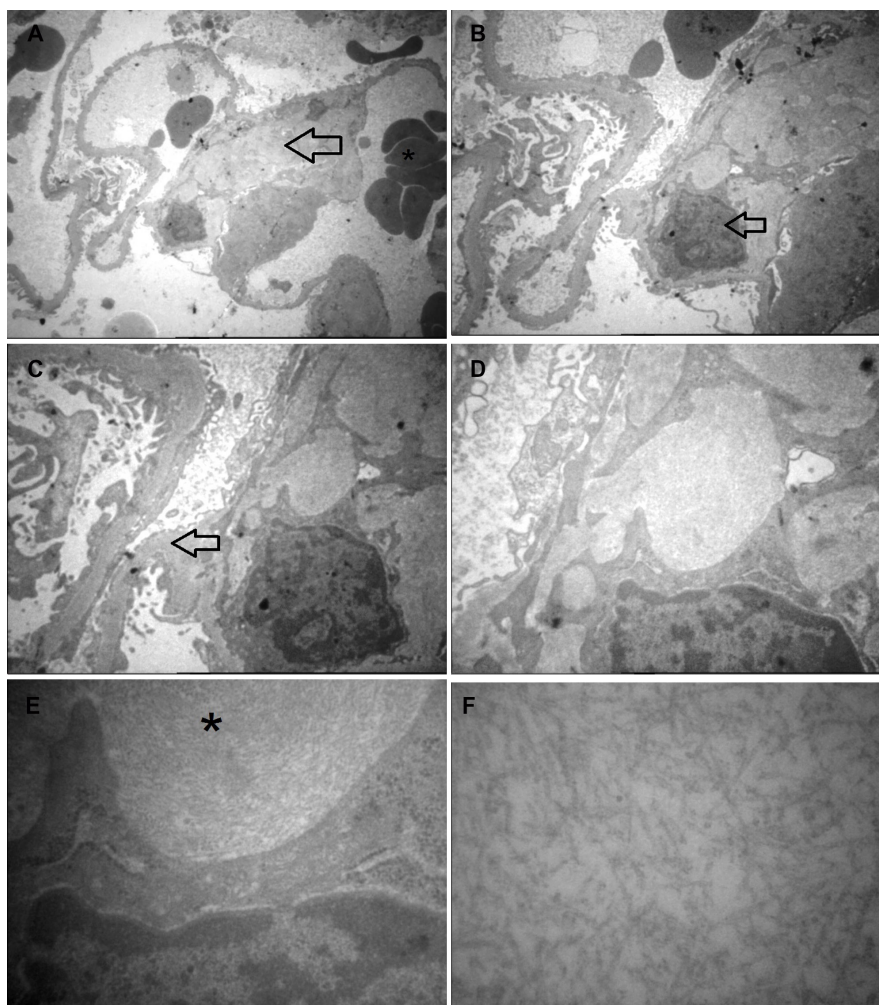
Assessment of the difference between the two main classes (A and B) showed that there



**Figure 2:** Photomicrographs of plastic sections of the kidney needle biopsy of renal amyloidosis shows depositions of pale blue homogenous amyloid material (asterisk) in A) the glomerulus, B) perivascular, C) peritubular, and D) interstitial areas by toluidine blue staining (magnification  $\times 400$ ).



**Figure 3:** Light microscopy findings of renal biopsies are shown. A) An asterisk indicates eosinophilic, homogenous, and acellular materials of amyloid depositions in the glomerulus stained with hematoxylin and eosin; original magnification  $\times 200$ . B) Weakly positive deposition of amyloid material on periodic acid-Schiff staining in the glomerular region (asterisk); original magnification  $\times 200$  is illustrated. C) Acellular, pale, eosinophilic, silver-negative material deposits within the glomerulus (arrow) by Jones silver staining; original magnification  $\times 200$  are shown. D) Weakly positive amyloid deposition on Masson staining in the glomerular region (asterisk); original magnification  $\times 200$  is illustrated. E) Amyloid deposition in the glomerular and mesangial region (arrow) stained with brick red or salmon color with Congo red; original magnification  $\times 200$  is shown.



**Figure 4:** Photomicrographs of electron microscopy of glomerular amyloidosis are illustrated. A) Homogenous electron-pale material deposition in the mesangial area (arrow) is shown. There are some red blood cells in the lumen of the capillary (asterisk); original magnification  $\times 2,700$ . B) The nucleus of the mesangial cell is visible (indicator); magnification  $\times 3,500$ . C) Homogenous pale material is deposited diffusely infiltrating glomerular basement membranes (arrow); magnification  $\times 4,500$ . D) Amyloid deposits extensively in the mesangial and intramembranous area; magnification  $\times 7,700$  are shown. E) Amyloid fibrils are indicated in the mesangial area (asterisk); magnification  $\times 16,700$ . F) Non-branching straight amyloid fibrils with a diameter of 8-12 nm are randomly oriented in the mesangial area; magnification  $\times 50,000$ .

was no significant difference in age and 24-hour urine volume, but the mean blood pressure significantly differed ( $P=0.003$ ). The proportion of patients with a history of hypertension (HTN) was significantly higher in class B (30.8% versus 78.6%,  $P=0.02$ ), and the patients in group B had a higher proportion of  $Cr > 1.5$  (64.3% versus 23.1%,  $P=0.03$ ). However, both groups were similar in terms of edema, hematuria, blood urea nitrogen, and the proportion of proteinuria (table 3). Assessment of the difference between the two main groups of amyloidosis grades (A and B) showed that there was no significant difference in age and mean blood pressure, but 24-hour urine volume was significantly different ( $P=0.01$ ) (table 3).

Clinical features with  $P < 0.20$  in univariate analysis (table 3) were used in the logistic regression analysis to assess the simultaneous

effect of classes and grades. The analysis showed that there was no significant association between the studied features and amyloid deposition classes, and grades. As shown in table 4, although the odds of higher glomerular classes among patients with HTN ( $OR=21.6$ ) and chronic kidney disease (CKD) ( $OR=2.77$ ) were higher, the reported probability was not statistically significant. Besides, the odds of higher grades among patients with rheumatoid arthritis (RA) ( $OR=1.04$ ) and patients with 24-hour urine protein  $< 3500$  mg was higher but without any significant association.

## Discussion

A comparison between the two new modified classifications of renal amyloidosis showed that a classification based on just glomerular

**Table 3:** Comparison of the variables among different groups of classes and grades

Clinical parameters	Classification based on glomerular classes			Classification based on grades			
	Class A	Class B	P value*	Grade A	Grade B	P value*	
	Mean±SD	Mean±SD		mean±SD	mean±SD		
Age	57.15±12.03	54.71±12.94	0.62	57.39±12.78	52.89±11.52	0.38	
Mean blood pressure	93.53±8.25	105.21±10.22	0.003	98.20±11.92	102.36±8.48	0.36	
24-hour urine volume(cc)	1892.50±768.15	1491.38±499.95	0.12	1845.00±700.50	1297.50±333.24	0.01	
	Number (%)	Number (%)	P value**	Number (%)	Number (%)	P value**	
Sex (male)	7 (53.8%)	9 (64.3%)	0.58	11 (61.1%)	5 (55.6%)	0.99	
Blood urea nitrogen (>23)	5 (38.5%)	7 (50%)	0.55	9 (50.0%)	3 (33.3%)	0.68	
Creatinine (>1.5)	3 (23.1%)	9 (64.3%)	0.03	7 (38.9%)	5 (55.6%)	0.45	
24-hour urine protein (>3500 mg)	6 (46.2%)	4 (28.6%)	0.44	9 (50.0%)	1 (11.1%)	0.09	
Urine protein with the dipstick (>2 plus)	9 (69.2%)	10 (71.4%)	0.99	13 (72.2%)	6 (66.7%)	0.99	
Nephrotic syndrome	7 (53.8%)	7 (50.0%)	0.84	10 (55.6%)	4 (44.4%)	0.70	
Hypertension	4 (30.8%)	11 (78.6%)	0.02	9 (50.0%)	6 (66.7%)	0.68	
Edema	9 (69.2%)	11 (78.6%)	0.68	12 (66.7%)	8 (88.9%)	0.36	
Hematuria	3 (23.1%)	5 (35.7%)	0.68	5 (27.8%)	3 (33.3%)	0.99	
Rheumatoid arthritis	3 (23.1%)	4 (28.6%)	0.99	3 (16.7%)	4 (44.4%)	0.18	
Chronic kidney disease	4 (30.8%)	9 (64.3%)	0.13	8 (44.4%)	5 (55.6%)	0.70	
Proteinuria	11 (84.6%)	13 (92.9%)	0.60	16 (88.9%)	8 (88.9%)	0.99	
Value of electron microscopy	Necessary	3 (25.0%)	1 (7.1%)	0.37	4 (23.5%)	0 (0%)	0.18
	Supportive	4 (33.3%)	4 (28.6%)		6 (35.3%)	2 (22.2%)	
	Non-helpful	5 (41.7%)	9 (64.3%)		7 (41.2%)	7 (77.8%)	
Tubular atrophy	Mild	10 (76.9%)	5 (35.7%)	0.03	10 (55.6%)	5 (55.6%)	0.99
	Moderate to severe	3 (23.1%)	9 (64.3%)		8 (44.4%)	4 (44.4%)	
Interstitial fibrosis	Mild	8 (61.5%)	8 (57.1%)	0.82	10 (55.6%)	6 (66.6%)	0.69
	Moderate to severe	5 (38.5%)	6 (42.9%)		8 (44.4%)	3 (33.3%)	

\*Based on independent t test; \*\*Based on chi square test

**Table 4:** Results of the logistic regression model on factors associated with higher classes and grades

Characteristics	Classification based on glomerular classes			Classification based on grades		
	OR	95% CI	P value*	OR	95% CI	P value*
Mean blood pressure	1.19	0.91-1.55	0.20	---		
Hypertension	21.65	0.43-1095.20	0.13	---		
Chronic kidney disease	2.77	0.17-45.36	0.47	---		
Creatinine (>1.5)	10.27	0.35-299.69	0.35	---		
Tubular atrophy (moderate to severe)	3.73	0.15-95.08	0.42	---		
24-hour urine volume (cc)	0.997	0.99-1.00	0.13	0.999	0.998-1.00	0.20
24-hour urine protein (>3500 mg)	---			0.54	0.10-3.04	0.48
Rheumatoid arthritis	---			1.04	0.16-6.93	0.97

\*Based on logistic regression analysis

involvement had a higher correlation with the clinical features. Diagnosis based on the EM investigation was either classified "necessary" or "supportive" in about 50% of the cases. Of the 27 cases, 51.9% of the patients were clinically diagnosed with nephrotic syndrome. Proteinuria and edema were the most prevalent clinical manifestations. Among inflammatory diseases, RA was the most common underlying disease followed by tuberculosis and familial Mediterranean fever (FMF). To the best of our knowledge, the present study is one of the most comprehensive EM-based studies on renal

amyloidosis in Iran.

Two modified pathological classification systems were used to assess the severity of renal amyloidosis. The results showed that classification based on glomerular involvement (classes), mean blood pressure, history of HTN, Cr>1.5, and severe tubular atrophy were significantly higher in class B. On the other hand, except for 24-hour urine volume, classification based on grades did not result in statistically significant differences in other clinical and laboratory variables between the two main grades. Therefore, it seems that a classification

based on just glomerular involvement (classes) has a higher correlation with clinical features than classification based on grades. Sen and colleagues were the first to assess the correlation between the clinicopathologic features and renal amyloidosis, based on which, they introduced a comprehensive class and grade system.<sup>9</sup> Their system was subsequently adapted and used in several other studies. In line with our findings, a recent study by Kalle and colleagues reported a good correlation between the class of glomerular involvement and clinical features.<sup>13</sup> In a cohort study, the severity of glomerular deposition was identified as a good prognostic factor for patient outcome prediction. It was found that 93% of the deceased patients had severe glomerular amyloidosis.<sup>15</sup> In the present study, we found that patients with severe glomerular amyloidosis (class B) had the highest levels of serum creatinine and severe tubular atrophy. This could be due to amyloid glomerular deposition significantly decreased the glomerular filtration rate and impaired the glomerular filtration barrier, which may enhance the progression of renal fibrosis. Overall, the severity of glomerular involvement had a good correlation with various clinical features, and was the most important factor affecting the loss of renal function in renal amyloidosis.

In the present study, we also assessed the clinical features of patients with renal amyloidosis. Based on the results, the nephrotic syndrome was the clinical diagnosis in about half of patients, and proteinuria and edema were the most common clinical manifestations. Our findings were consistent with the outcome of several other studies. In a study conducted in the United States, Alexander and colleagues reported that all patients with amyloidosis presented proteinuria, and 47% of them were clinically diagnosed with nephrotic syndrome.<sup>16</sup> In China, from a total of 47 samples, Luo and colleagues reported that 57.4% of the patients with renal amyloidosis had edema.<sup>17</sup> A recent study revealed that proteinuria was the most common renal manifestation, and 70% of the patients had a nephrotic syndrome.<sup>18</sup> Two other studies reported almost similar results.<sup>13, 19</sup> In terms of the underlying diseases, HTN, CKD, and RA were the most common, and more than half of the patients had a history of consuming cardiovascular drugs, including antihypertensive agents. This finding was in line with other studies that reported HTN as a common underlying disease in renal amyloidosis. In a case series conducted in the United States, about half of the patients with renal amyloidosis had HTN, which is close to our results (55.6%).<sup>16</sup> In patients

with HTN, controlling blood pressure with antihypertensive treatment protected them from renal dysfunction.<sup>20, 21</sup> However, another study showed that HTN did not affect renal survival.<sup>22</sup> Moreover, a previous study reported hypotension as one of the features of amyloidosis.<sup>23</sup> The exact mechanism of hypotension is not clear, nonetheless, it can be due to the juxtaglomerular apparatus involvement by amyloidosis followed by the suppression of the renin-angiotensin-aldosterone system. Further research is required for a better understanding of these conflicting results.

Amyloidosis can be a complication of chronic inflammatory diseases such as RA, tuberculosis, and FMF. In our study, among the inflammatory diseases, RA was the most common disease followed by tuberculosis and FMF. Labib and colleagues reported a 12.5% prevalence rate of RA in patients with renal amyloidosis compared to 25.9% in our study.<sup>24</sup> A study conducted in the UK on 374 patients reported RA prevalence rate at 33%.<sup>25</sup> Along the same lines, an Italian study among 373 patients with renal amyloidosis reported RA as the common underlying disease.<sup>26</sup> Reversely, patients with RA are also at risk of developing amyloidosis.<sup>27</sup> The reported prevalence of amyloidosis in RA patients varies in different studies due to different study populations and diagnostic strategies.<sup>28, 29</sup> FMF is another predisposing inflammatory disease for amyloidosis, which had a low prevalence in our study. This could be due to regional characteristics of the disease or the physicians' diagnostic skills. The reported prevalence rate of FMF in most studies was higher than in our results. For instance, an EM-based study among the Egyptian people over a period of 25 years showed that 34% of the patients had FMF.<sup>24</sup>

We found that the diagnosis rate of renal amyloidosis was 0.94%, and it mainly occurred in the male patients (59.3%). This was in line with the findings of other studies, which concluded that the disease chiefly occurs in the male population. However, diverse male-to-female ratios (from 1 to 1.7, 2.3, and 3.3) have been reported in previous studies.<sup>26, 13, 18, 24</sup> Clearly, further research is required to understand the reason behind such variations. In terms of the diagnosis rate, a review article indicated that the male sex increased poor renal prognosis.<sup>30</sup> The diagnosis rate in our study (0.94%) was lower than in some other studies. For example, studies conducted in Japan and Egypt reported a diagnosis rate of 1.7% and 2.97%, respectively.<sup>14, 24</sup> The mean age of the patients with renal amyloidosis in our study was 55.9±12.3. Two other studies reported a similar mean age (40.9 and 50),<sup>24, 25</sup> whereas



some others reported different mean age.<sup>14, 16, 18</sup> In general, amyloidosis with renal involvement occurs mainly in adolescents, and is rare in young people and children.<sup>25</sup>

Some limitations of our study should be noted. The quality of the hospital records was one of the limitations, since some physicians' notes were un-descriptive or incomplete. For instance, some pathology requisition forms were not correctly filled out by the staff at the hospital ward. Another limitation was related to the immunohistochemistry (IHC) study at our center, which was not routinely performed for all patients to identify the subtypes of amyloidosis. It is recommended that future studies determine the correlation between EM and IHC study in renal amyloidosis. On a more positive note, we found that plastic sections from resin blocks stained with toluidine blue, which helped to determine amyloid deposition in various parts of the kidney by detecting the pale blue homogenous material. Very few studies have been conducted on the role of this staining procedure for the diagnosis of amyloidosis. Future studies in this area are strongly recommended to elucidate the diagnostic strength of toluidine blue staining for the diagnosis of amyloidosis.

## Conclusion

The findings of the present study indicated that EM investigation plays an important role in the diagnosis of renal amyloidosis. EM in conjunction with LM investigation with Congo red staining is recommended to prevent misdiagnosis in patients with clinical suspicion of renal amyloidosis. Among different pathological features, the severity of glomerular amyloid depositions had a higher rate of association with clinical features, such as high blood pressure and creatinine. To assess the subtypes of renal amyloidosis, it is recommended to perform studies with a larger sample size to determine the correlation between EM and IHC study.

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