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IMMUNOFLUORESCENCE STUDY OF CHILDHOOD RENAL AMYLOIDOSIS*

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SUMMARY: Tınaztepe K, Güçer KŞ (Nephropathology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Immunofluorescence study of childhood renal amyloidosis. Turk J Pediatr 34:5-14, 1992.

In this paper, the findings of immunofluorescence (IF) studies of 57 patients with childhood biopsy-proven renal amyloidosis are presented. All specimens were investigated by the direct IF technique and the simultaneous use of antisera to human IgG, IgM, IgA, fibrinogen and C3. Antisera to C1q, C4, HbsAg, IgE (in each of ten cases), kappa and lambda light chains of immunoglobulins (Igs) and albumin (in each of five cases) were also used. AA type amyloidosis was determined in all patients by Wright's potassium permanganate reaction. In thirty-four of these patients (60%), Familial Mediterranean Fever (FMF)was found to be the underlying disease for renal amyloidosis.

In 39 cases (68.5%), renal biopsy showed positive fluorescence staining while in 18 cases (31.5%), fluorescence staining was negative. The immunofluorescence pattern of glomerular deposits was neither granular nor linear but large isolated or confluent masses which were located in the mesangium and in the capillary walls, and were similar in all cases whatever the antisera used. The areas showing immunofluorescence staining almost corresponded to the locations of amyloid deposits. Immunoreactants showed various combinations of deposition with the exception of IgE, HbsAg and albumin antisera which yielded continuously negative reactions. C3 was the immunoreactant most commonly encountered. Kappa and lambda light chains of Igs were demonstrated in one of five biopsy specimens tested. Although it was not diagnostic, this IF pattern was found to be rather characteristic.

Demonstration of immunoglobulins and other components of the humoral immune system is not a rare occurrence in renal amyloidosis, and passive absorption of plasma proteins does not simply explain these immunohistologic findings. *Key words: immunofluorescence, immunoglobulins, renal amyloidosis, childhood.*

Amyloidosis is a disorder of protein metabolism characterized by the accumulation of abnormal extracellular deposition of an eosinophilic and hyalin material which is recognized on light microscopy by its distinctive staining properties and its fibrillary structure[1]. Despite great advances in the characterization of the chemical nature of amyloid fibrils, the precise mechanism of amyloid fibril formation and deposition remains unsettled. In recent years, two predominant types of amyloidosis, AA and AL, have been histochemically recognized. Immunoglobulin light chain-related deposits (AL) have been found to be the major

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components of amyloid fibrils obtained from patients with primary amyloidosis whereas the presence of amyloid protein A has been found to be associated with amyloid fibrils of the AA type in secondary amyloidosis[2-4].

Several authors have reported the presence of immunoglobulins, complement components and fibrinogen in amyloid deposits, and these proteins have been postulated to play a role in the pathogenesis of amyloidosis[5-9]. However, little systematic data are available in the literature regarding immunofluorescence findings and immunohistologic patterns in human renal amyloidosis.

This study describes direct immunofluorescence findings in childhood renal amyloidosis using fluorescein-labeled antisera specific for various human immunoglobulins, complement components and other serum proteins.

Material and Methods

The study population consisted of 57 patients with tissue-diagnosed renal amyloidosis. There were 33 males and 24 females, with a male/female ratio of 1.4:1. The ages of the patients ranged between five and eighteen years. Significant clinical data of these patients are listed in Tables I and II. Fifty-five needle and two open-biopsy specimens of 57 cases were studied.

Renal tissue samples were processed for conventional light and immunofluorescence microscopic studies. Representative sections contained at least three glomeruli. Specimens were fixed in Duboscq-Brasils solution and embedded in paraffin for light microscopic examination. Sections cut at 2-4 microns were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), Jones Silver, Masson's trichrome and trichrome-L stains. The tissues were sectioned at 6 microns and stained with the Buchtler modification of the Congo red stain. The presence of amyloid was confirmed in the Congo red stained sections by demonstration of an apple-green birefringence under polarized light.

The differentiation between AA and AL type amyloid was made by Wright's potassium permanganate reaction[10] and evaluating the presence or absence of any recognized associated disease.

For study by immunofluorescence microscopy, biopsy material obtained from all patients was rapidly frozen in a mixture of CO_2 and acetone and sectioned at 2 microns in a Tissue-TEK II Cryostat at −20 °C, and incubated with fluorescein-conjugated antisera specific for human IgM, IgA, IgG, fibrinogen and C3 (Behringwerke AG Laboratories, Marburg). Antisera to C1q, C4, HbsAg and IgE (in each of ten cases) were also used. Staining with antisera for kappa and lambda light chain of Igs and albumin was accomplished in each of five cases. Sections were immediately examined under an ortho-lux Leitz microscope with an epifluorescent lamp.

Results

All specimens demonstrated evidence of amyloid deposition both histochemically and histopathologically. They showed green birefringence of Congo red positive tissue sections when examined by polarized microscopy (Figs. 1, 2). The secondary (AA) type of amyloidosis was determined in all cases by the use of the potassium permanganate ($KMnO_4$) reaction. The glomeruli mostly contained amyloid deposits whereas the other elements of the nephron such as the tubular basement membranes and vessels were rarely affected (Table III). In general, Familial Mediterranean Fever (FMF) was the condition most commonly associated with renal amyloidosis in the cases we studied (60%) (Tables I, II).

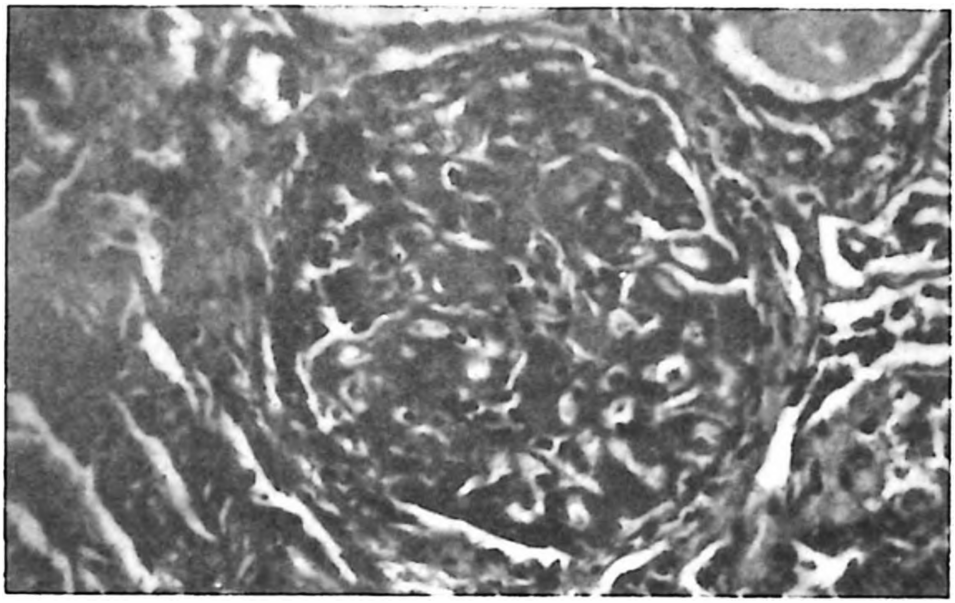

Fig. 1: Diffuse amyloid involvement of glomerular capillary wall (lower left) (Congo red × 450).

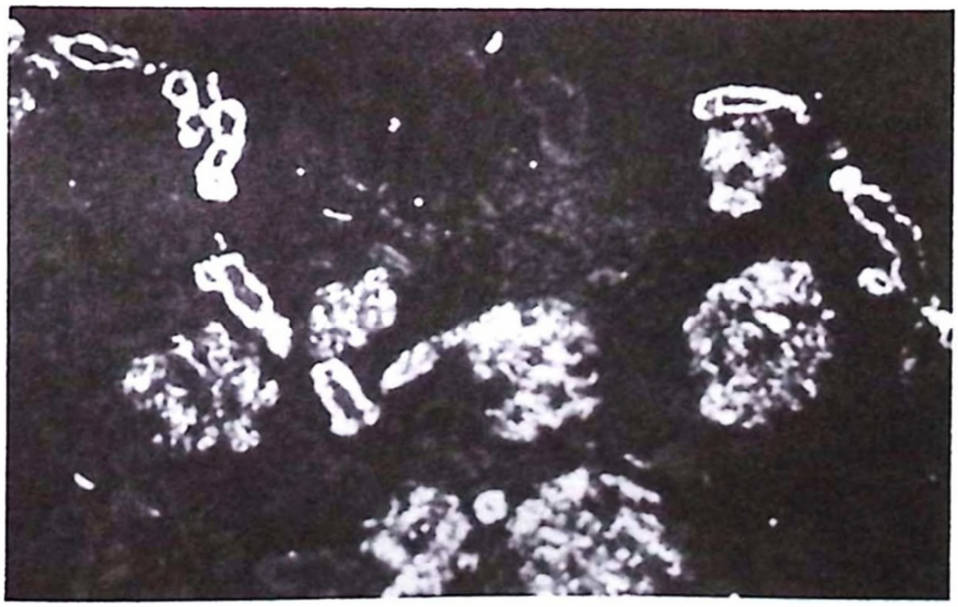

Fig. 2: Diffuse amyloid involvement of glomeruli tubuli and vessels under polarized light (Congo red × 90).

TABLE I: A Summary of Clinical Data of 39 Patients with Renal Amyloidosis Showing Positive Staining With Immunoreactants

No. of Patient	Age / Sex (yrs)		Associated Condition	Serum Creatinine (mg/dl)	Proteinuria (g/24 hr)
1	6	F	UD	0.4	4.6
2	5	F	FMF	0.6	2.0
3	14	M	MPGN	1.8	4.0
4	12	M	FMF	0.6	4.4
5	13	F	FMF	0.6	4.4
6	12	M	FMF	0.4	4.2
7	12	F	FMF	0.6	4.0
8	14	F	UD	6.0	2.6
9	16	F	FMF	0.4	3.3
10	13	F	FMF	0.8	0.5
11	14	F	FMF	0.8	4.4
12	5	F	FMF	0.6	2.6
13	9	M	Hodgkin's L.	0.6	0.1
14	12	M	UD	0.6	3.6
15	13	M	FMF	0.5	1.0
16	11	M	FMF	0.5	2.4
17	12	M	FMF	0.8	0.4
18	18	M	FMF	0.5	2.5
19	11	M	FMF	0.6	1.0
20	13	F	Tuberculosis	0.3	3.0
21	15	M	UD	0.6	2.6
22	13	M	FMF	0.6	1.3
23	10	M	FMF	0.5	1.7
24	15	M	FMF	0.3	6.0
25	15	F	Hodgkin's L.	0.5	1.0
26	11	M	Bronchial Asthma	0.5	2.7
27	14	M	FMF	1.0	4.9
28	11	M	FMF	0.3	3.3
29	14	F	UD	1.8	2.1
30	15	M	UD	1.0	3.2
31	12	M	FMF	0.8	11.0
32	18	M	UD	1.0	1.6
33	9	F	JRA	0.4	5.0
34	15	F	UD	0.8	–
35	13	M	UD	0.8	5.0
36	14	M	Tuberculosis	0.8	3.9
37	11	M	FMF	0.5	10.0
38	7	F	FMF	0.5	4.8
39	12	M	FMF	0.5	5.4

UD : Undetermined.
FMF : Familial Mediterranean fever.
MPGN: Membranoproliferative glomerulonephritis.
JRA : Juvenile Rheumatoid Arthritis.

TABLE II: A Summary of Clinical Data of 18 Patients with Renal Amyloidosis Demonstrating Negative Immunofluorescence

No. of Patient	Age / Sex (yrs)		Associated Condition	Serum Creatinine (mg/dl)	Proteinuria (g/24 hr)
1	12	F	Cr.Pyelonephritis	–	–
2	9	M	UD	–	–
3	13	M	FMF	1.0	4.0
4	8	M	FMF	1.2	2.5
5	13	F	UD	0.8	4.5
6	13	F	FMF	1.0	2.0
7	12	F	FMF	0.6	4.0
8	11	F	FMF	0.6	3.5
9	12	F	FMF	0.6	3.8
10	12	M	FMF	0.8	6.7
11	17	M	FMF	1.2	2.5
12	15	M	FMF	3.8	–
13	15	M	JRA	0.6	5.0
14	13	M	UD	0.2	4.1
15	8	F	FMF	0.6	2.9
16	13	F	FMF	7.0	4.8
17	7	M	JRA	0.4	2.5
18	11	F	UD	0.6	3.1

UD : Undetermined.
FMF: Familial Mediterranean fever.
JRA : Juvenile Rheumatoid Arthritis.

TABLE III: Localization of Immune Reactants in the Glomeruli of 39 Patients With Renal Amyloidosis

Glomerular Location	Number of Patients
Mesangium only	16
Mesangium + GBM	9
Mesangium + GBM + TBM	6
Mesangium + TBM	4
Mesangium + Vessels	2
Mesangium + GBM + TBM + Vessels	2

GBM: Glomerular basement membrane.
TBM : Tubular basement membrane.

On immunofluorescence microscopy, there was no staining in the specimens of 18 of 57 patients (31.5%). All biopsy material was classified into two groups. Group I consisted of the biopsies of patients performed before 1981 and studied with monospecific antisera to IgG, IgA, IgM C3 and fibrinogen. The specimens of 42 patients were included in this group, and 16 (35%) of them showed no staining. Group II consisted of the specimens of 15 patients which were studied with additional specific antisera. Tests for immunofluorescent staining of HbsAg, IgE and albumin were all negative. Positive fluorescence was demonstrated in one of five cases tested with kappa and lambda light chain of Igs. Both groups showed that C3 was the immunoreactant most commonly encountered in the amyloid deposits except for C1q in the second group (Tables IV, V). Of the staining patterns, C3, IgM and fibrinogen were the most common combination patterns.

TABLE IV: Distribution of Deposition of Immunoreactants in Biopsy Specimens of Group I Patients (42)

Immunoreactant	No. of Cases Studied	No. of Positive Stainings
C3	42	21 (50.0%)
Fibrinogen	42	20 (47.4%)
IgM	42	18 (42.8%)
IgA	42	9 (21.4%)
IgG	42	7 (16.6%)

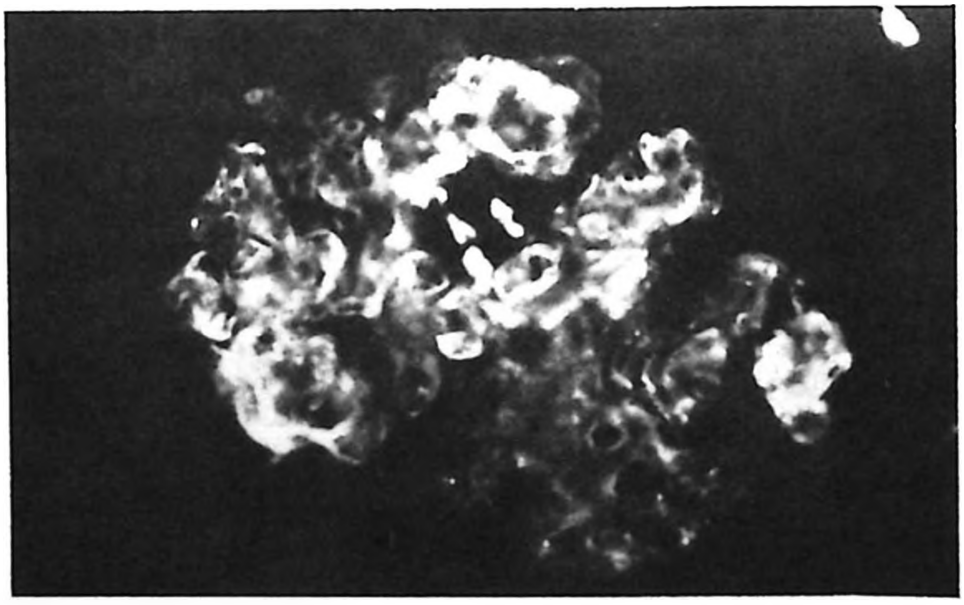

Fig. 3: Large, confluent mass of IgM in the mesangium; typical pattern of fluorescent staining in renal amyloidosis (fluorescence microscopy × 450).

TABLE V: Distribution of Deposition of Immunoreactants in Biopsy Specimens of Group II Patients (15)

Immunoreactant	No. of Cases Studied	No. of Positive Stainings
C3	15	10 (66.6%)
IgM	15	8 (53.3%)
Fibrinogen	15	8 (53.3%)
IgG	15	5 (33.3%)
IgA	15	2 (13.3%)
C1q	10	8 (80.0%)
C4	10	3 (30.0%)
HbsAg	10	0 (00.0%)
IgE	10	0 (00.0%)
Lambda light chain	5	1 (20.0%)
Kappa light chain	5	1 (20.0%)
Albumin	5	0 (00.0%)

Immunofluorescence of amyloid deposits was rather characteristic whatever the antisera used. Positive fluorescence in the specimens was mainly in the mesangium of the glomeruli (Figs. 3, 4). Glomerular depositions formed characteristically large isolated or confluent round masses which were localized to

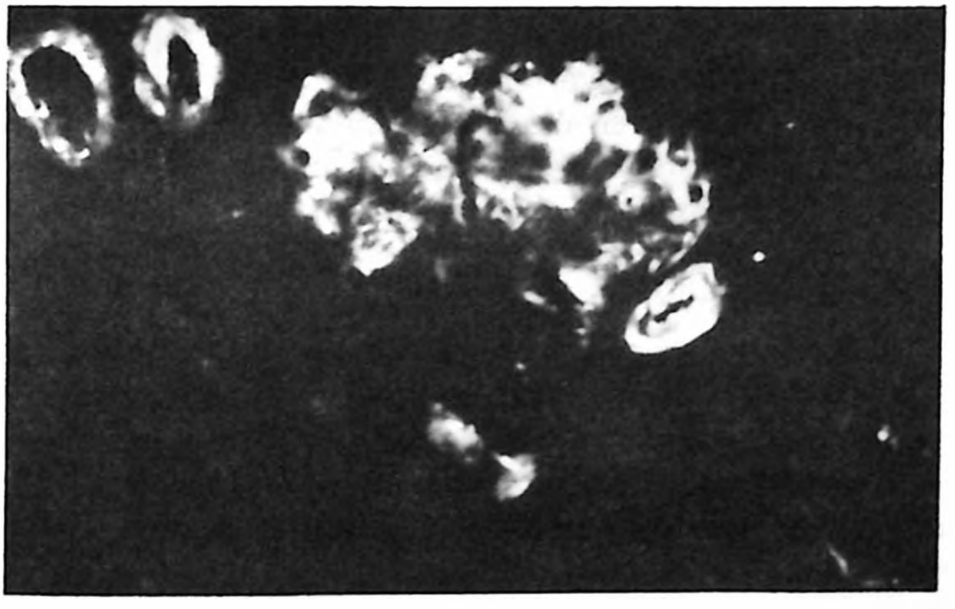

Fig. 4: Diffuse and homogenous deposition with C1q in the glomerulus. There is also staining of preglomerular arterioles (fluorescence microscopy × 350).

mesangial areas and capillary walls (Figs. 4, 5). A few specimens showed staining along the glomerular and tubular basement membranes. There was also staining of tubular casts and arterial walls (Fig. 4).

The localization of immunoreactants and amyloid deposits observed by light microscopy evidenced good conformity.

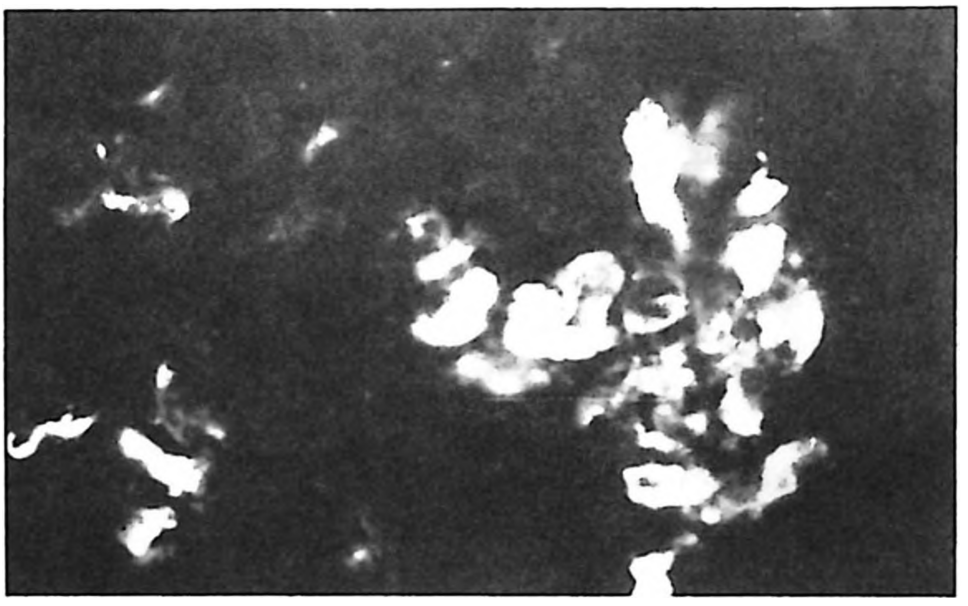

Fig. 5: Nodular deposition of C3 in the mesangium in a glomerulus (fluorescence microscopy × 450).

Discussion

In this study, it was found that FMF was the underlying desease in the majority of renal amyloidosis cases studied. This result correlated well with Turkey's geographic location. With the advances in treatment of infectious diseases, FMF has recently replaced suppurative conditions as a cause of amyloidosis in our country[7,11-13] (Tables I, II). Of 57 cases in this study, 13 (22.8%) showed neither clinical nor laboratory findings of any recognizable underlying disease of renal amyloidosis. These patients were thought to be phenotype II, because AA type renal amyloidosis might have preceded the acute attacks and was possibly the sole manifestation of FMF[14].

The presence of immune complexes in glomerular capillary walls provides substantial evidence for an immunological mechanism in renal disease[15]. In several studies, deposits of immunoglobulins and other components of the humoral immune system have been demonstrated immunohistologically in both primary and secondary amyloidosis[5,9-16]. In many of these reports, amyloid deposits have been demonstrated to localize primarily in mesangial areas. Our study confirmed previous studies. The other elements of the nephron were also demonstrated to have been involved in a number of our patients (Table III). The

distribution of mesangial amyloid fluorescent deposits may resemble immune-complex glomerulonephritis but differs morphologically. The fluorescence pattern in amyloidosis is nongranular, nonlinear and homogenous, which is quite different from the granular pattern of immune complex diseases and the linear pattern of anti-glomerular basement membrane antibody disease (Fig. 4).

Glenner et al[17] reported that a relationship existed between amyloid and light chains of Igs and showed that it was possible to create amyloid fibrils from certain Bence-Jones proteins by proteolytic cleavage in vitro. The major protein found in some amyloid fibrils is a portion of an immunoglobulin (Ig) which might point to its being causally involved in renal amyloidogenesis[7].

In recent years, it has been determined that the formation and depositions of amyloid fibrils depend on the presence of an autologous protein precursor which is either a protein circulating or produced locally with proteolytic cleavage by macrophages, endothelial cells, etc[2-4]. This could be a circumstantial, immunological role played by glomerular epithelial cells in renal amyloidogenesis. On the other hand, staining with antisera to IgE, albumin and transferrin has been reported to be consistently negative[6-8], and immune complexes have been demonstrated in some AA and immunocytic amyloidosis cases[9-13]. Due to these results, passive nonselective absorption of plasma proteins cannot account for the immunofluorescence findings observed in a few cases of FMF[9-13].

In this study, we observed positive fluorescence in 68.5 percent of all cases, which is in accordance with the study of Jerath et al[8] and Orfila et al[9]. In most of our cases, C3 was found with variable amounts of IgG, IgA and IgM (Tables IV, V). C3, IgM and fibrinogen were the most commonly encountered immunoreactants in the group I specimens we studied. The biopsies of group II, of which additional C1q stainings were applied, yielded about the same results, but C1q (80%) was observed more frequently than C3 (66%), IgM (53.3%), IgG (33.3%) and others (20.0%-13.3%) (Table V). A positive reaction for IgG was noted in 12/57 (21.0%) of overall cases. This value differs slightly from that reported by Müftüoğlu et al[7], Jerath et al[8] and Orfila et al[9].

The staining patterns were homogeneous, non-granular and nonlinear (Figs. 4, 5). The immunofluorescent deposits were observed to form round and confluent masses, mostly in the mesangium. They were also observed to be present along the glomerular and tubular basement membranes, on the vascular walls and in the tubular casts, similar to results mentioned in the literature[7-9] (Table III). The immunofluorescence patterns were rather characteristic and similar in all patients except for minor differences related to primary disorders. The lambda and kappa light-chains of Igs were found in 1/5 (20%) of our patients but albumin and IgE were consistently absent in the tested cases. In view of these biologic data, it is possible that these immunoreactants may be involved in the development of renal

lesions in amyloidosis though their exact role is not clear. It is, therefore, necessary to perform further detailed investigations to elucidate the true mechanism with regard to the presence of humoral components of the immune system and the roles they play in renal amyloidogenesis.

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