ORIGINAL ARTICLE

Clinicopathological Study of Renal Amyloidosis

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Abstract

Study included 13 cases of renal amyloidosis.Oedema, feet and face was the commonest manifestation (100%), two patients (18.18%) also presented with loose motions, ascites and pain in abdomen and one patient had ankylosing spondylitis and cervical spondylitis. On clinical grounds only one case was diagnosed as primary amyloidosis of light chain type, who presented initially with cervical lymphadenopathy and 4 years later with nephrotic syndrome. About 72.72% cases had some chronic disease in the terms of tuberculosis, ankylosing spondylitis, chronic ulcerative colitis, lepromatous leprosy, rheumatoid arthritis and one patient had carcinoma caecum. Congo red stain was positive in both, light chain deposit disease (LCDD) and amyloidosis but polarizing microscope showed mixed birefringence (red, green, yellow) only in amyloidosis. In AFOG and PAS stain, amyloid appeared negative, only peripheral portion revealed blue and pink staining and central area appeared as cutout spaces. Congo red and methyl violet stains and potassium permanganate treatment was not helpful in distinguishing AL amyloidosis from secondary amyloidosis. Hence immunohistochemistry and myeloma profile is a must. It might be possible that in light chain amyloidosis, treatment with methotrexate and prednisolone may improve survival.

Key Words : Amyloidosis, Tuberculosis, Light Chain Deposition Disease.

Introduction

Amyloidosis is defined as a group of chronic infiltrative disorders that have in common, beta pleated sheet configuration on X-ray diffraction examination and a fine, non branching fibril on electron microscopy (1). Histologically it is characterized by deposition of hyaline fibrillar material in interstitial tissue. Chemically fibrils of amyloid are heterogenous. Light chain immunoglobulins are seen in primary amyloidosis, amyloid associated protein is seen in chronic inflammatory diseases and Familial Mediterranean Fever, transthyretin is found in familial amyloid neuropathy and senile cardiac amyloidosis ; apolipoprotein, cystatin C, lysozyme and fibrinogen in familial amyloidosis ; b4 protein in Alzheimer disease, trisomy 21 and dutch cerebral amyloid angiopathy. Islet amyloid polypeptide is seen in type II diabetes mellitus, calcitonin in medullary carcinoma thyroid and atrial natriuretic peptide in isolated atrial amyloidosis (2). Besides this, amyloid may consist of lactoferrin in corneal amyloidosis and b2microglobulin after haemodialysis.

Diagnosis of amyloidosis is confirmed by staining the section with Congo red and examining the stained section in polarized light. In ordinary light, amyloid appears orange to red and in polarized light, it gives yellow-green birefringence. Ultrastructural examination for demonstrating fibrillary structure is rarely needed (3).

Some non traumatic techniques may also help in diagnosis. Serum paper electrophoresis discloses monoclonal protein in two third cases and if combined with urine electrophoresis, it discloses para protein in 86% cases. In splenic amyloidosis, peripheral blood examination shows Howell-Jolly bodies (4). Amyloidosis involves various parenchymal organs e.g. kidney, liver, spleen, rectal tissue, pancreas, adrenal glands and mesenchymal organs e.g. cardiac muscle, adipose tissue, fascia and tendons etc. For demonstrating the chemical

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nature of amyloid fibril, immunohistofluorescence on frozen section by using monoclonal antibodies to various fibril proteins may be used (2).

Aim of the present study was to see the clinicopathological features in renal amyloidosis. The study also attempted the various routine and special stains in its diagnosis.

Material and Methods

Thirteen cases of renal amyloidosis were detected in the 5 year period (January 1997 to February 2002). All cases were referred from the Department of Nephrology, Sir Sunderlal Hospital, Banaras Hindu University. Detailed clinical history was sent by clinicians. Renal biopsy was sent in 10% formalin. Routine paraffin blocks were prepared and 2m thick sections were stained with haematoxylin and eosin, Congo red stain (Bennhold technique) methyl violet (Lendrum's technique) PAS stain as described by Culling (5). Acid Fuschin Orange G (AFOG) stain as described by Zollinger and Mihtasch (6). In two cases, primary amyloidosis was suspected clinically. In these cases, serum and urine paper electrophoresis along with urine Bence Jones proteins were done (7). Radiological tests to find lytic lesions and osteoporosis in bone and sternal marrow for plasma cells were also done. Acidified potassium permanganate treatment was done to see whether amyloidosis was of AL or AA type (8). Cases were classified as primary amyloidosis, when no known disease was associated or secondary when it was associated with a known disease.

Results

Thirteen cases of renal amyloidosis were diagnosed histologically. They constituted 2% of the total cases of renal biopsies. All the cases except one presented to Nephrology Department as nephrotic syndrome.

There was marked male predominance (M:F ratio 12:1). Age ranged from 18 to 75 years with mean age of 43.15 years. Duration of renal manifestations varied in majority of cases from 20 days (carcinoma caecum) to 1.5 years (ulcerative colitis). Clinical features obtained in 11 cases, revealed oedema of feet and face as the commonest presentation (100%), followed by hypotension (36.36%), pain abdomen, diarrhoea and vomiting (18.18%). One patient of AL amyloidosis presented with cervical lymphadenopathy of 4 years duration along with carpal tunnel syndrome and oedema of feet for last 1 year (Table 1). Cervical lymph node biopsy done about 4 years back revealed features of amyloidosis. The patient underwent some treatment resulting in regression of cervical lymphadenopathy. About one year back, he developed pain in abdomen which revealed mesenteric lymphadenopathy on ultrasound examination. Three months back, he developed carpal tunnel syndrome and odema of feet and face. Lymph node biopsy revealed deposition of fibrillar hyaline material surrounded by multinucleated giant cells at the periphery and plenty of plasma cells. Lymph node architecture was completely lost. Biopsy of tendon and fascia showed hyaline deposits. Both the biopsies revealed patchy positivity for Congo red and methyl violet stain. Urine electrophoresis revealed mild 'M' peak in g region and positive Bence Jones protein test in urine. Bone marrow examination showed 15% plasma cells. No lytic lesion was present.

History of associated diseases was obtained in 72.72% of which tuberculosis was found in 27.27% (Table II). One young male patient aged 18 years was suffering from ankylosing spondylitis and cervical spondylosis for last 8 years and was on some ayurvedic drug. About 2 months prior to admission, he developed oedema of feet and face. Urine examination revealed proteinuria with haematuria and leucocytoturia.

One male patient aged 43 years, was having frequent attacks of jaundice and `finally developed nephrotic syndrome. Another male patient of 45 years was suffering from lepromatous leprosy for the last 10 years and had nephrotic syndrome for last 6 months.

One young male patient aged 22 years was having chronic ulcerative colitis. He developed nephrotic syndrome 1½ years back and underwent treatment following which he was relieved of his symptoms. One month back again he developed nephrotic syndrome with ascites, anasarca and rheumatoid arthritis. His kidney biopsy revealed marked hyaline thickening of intertubular capillaries and tubular basement membrane. There was duplication of tubular basement membrane in addition to the deposition of amyloid in mesangium and glomerular basement membrane. Amyloid was strongly positive for Congo red and methyl violet.

One 75 years old female had a history of pain in abdomen and diarrhea for last 2 months. She was

diagnosed as a case of carcinoma caecum. About 20 days back, she developed oedema of feet and face. Kidney biopsy was done, which revealed severe nodular fibrillar deposition of amyloid in mesangium, glomerular basement membrane and interstitium and hyaline globules in cytoplasm of the proximal tubule. Hyaline material was positive for Congo red stain but negative for methyl violet stain and it was dark blue on AFOG trichrome stain.

Hence, in nutshell, 72.72% cases were diagnosed as secondary amyloidosis and 28.28% patients were diagnosed as primary amyloidosis, of which 1 patient of light chain amyloidosis was detected.

Reports of blood urea and serum creatinine were provided in 9 cases. In 2 cases blood urea and serum creatinine were said to be within normal range but the exact values were not known. In 22.22% cases, patients had raised blood urea and serum creatinine while in the rest they were in normal range. Clinically also these 2 patients had features of chronic renal failure with normal sized kidney.

Twenty four hour urinary protein report was provided in 7 cases. In 4 cases, it was said to have nephrotic range proteinuria but the exact value was not provided. Only 1 patient had non-nephrotic range proteinuria and the rest had urinary protein more than 4.87 grams/24 hours with mean value of 7.63 grams/24 hours (Table III). Urine examination report was provided in 10 cases also supported the predominance of nephrotic range proteinuria. One patient had 2+ proteinuria and the rest had 3+ to 4+ proteinuria. Leucocytoturia containing mononuclear cells and neutrophils was found in 80% cases (Table IV).

Histologically predominant finding in the kidney was irregular, focal ring like hyaline deposits in a few glomerular capillary loops (84.611%) accompanied by hyaline mesangial cell deposit in 61.53% cases. In 2 cases, nodular mesangial deposits mimicking nodular glomerulosclerosis (NGS) of diabetes mellitus and light chain disease were noted. In 46.15% cases fibril formation was found. In 2 cases, focal mesangial cell proliferation was noted along with hyaline deposit. These cases showed red deposit in mesangium in AFOG stain (Table V). One of these cases was secondary to carcinoma caecum and another case was associated with tuberculosis.

Tubular changes included vacuolar degeneration (30.76%) and thyroidization (46.14%). Amyloid deposition in tubular basement membrane (Fig. 1) was seen in only 2 cases (Table VI). Amyloid deposition in capillary and arterioles was seen in only 30.7%. Medium sized blood vessels showed patchy amyloid deposition in one third cases (Table IV).

Patchy severe mononuclear cell infiltrate with neutrophils were frequent in interstitium (61.5%) (Table VI). Some special stains e.g. Acid Fuschin Orange G (AFOG), PAS, Congo red, methyl violet stains were done to see the colour of amyloid. In majority cases amyloid in AFOG stain, appeared pale blue surrounded by dark blue membrane (Fig. 2). In 2 cases, surrounding mesangium area appeared red in colour and these cases also had mesangial cell proliferation.

On PAS stain, amyloid appeared pale pink in majority of cases (Fig. 3) and only 33.33% cases showed focal positivity. On methyl violet stain, only two cases of amyloid did not show positivity and both were elderly patients aged 75 years and 65 years. On biopsy predominantly nodular type of amyloid with fibril formation and glomerulosclerosis was seen and the specimen was positive for Congo red stain (Table VII). In Congo red stain all amyloid were positive.

Polarized microscopy of Congo red stained section revealed mixture of yellow green and red birefringence rather than diffuse green birefringence as reported in literature. Five cases of light chain disease which also showed mild congophlia revealed no birefringence in polarized light (Table VIII). Hence polarized microscopy should be taken as confirmatory test for amyloidosis.We tried for acidified potassium permanganate pretreatment which destroy secondary amyloidosis but retain light chain and transthyretin amyloidosis, but all the fine sections desquamated during staining.

Table I
Clinical Manifestations of Renal Amyloidosis

Clinical Features	No. (Total cases - 11)	Percentage
Edema of feet and face	11	100.0
Hypotension	4	36.36
Normal B.P.	6	54.54
Pain abdomen, loose motions, vomiting	2	18.18
Renal failure	2	18.18
Cervical LN, enlargement, carpal tunnel syndrome, edema of feet	1	9.09

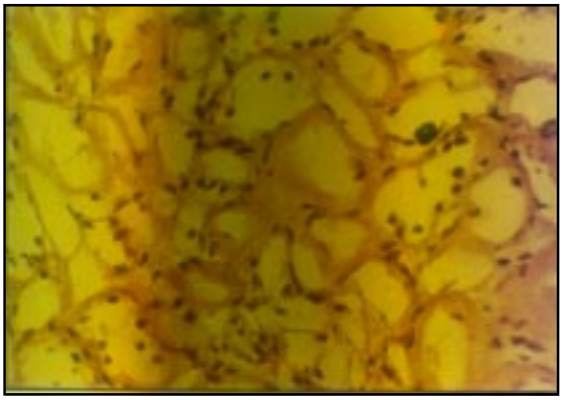

Figure 1. Shows hyaline like amyloid deposit in tubular basement membrane (H&E ´ 500)

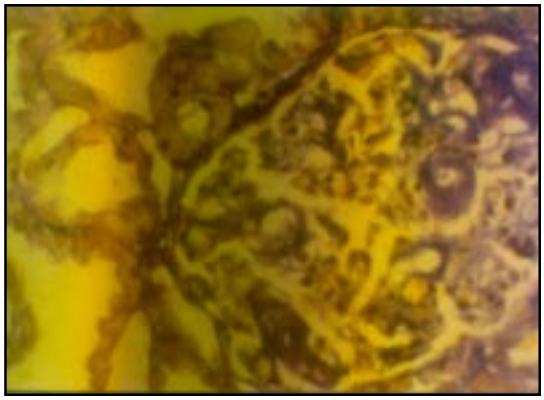

Figure 2. Amyloid material is appearing as blue in GBM and mesangium in Acid Fuchsin Orange G stain (AFOG ´ 500)

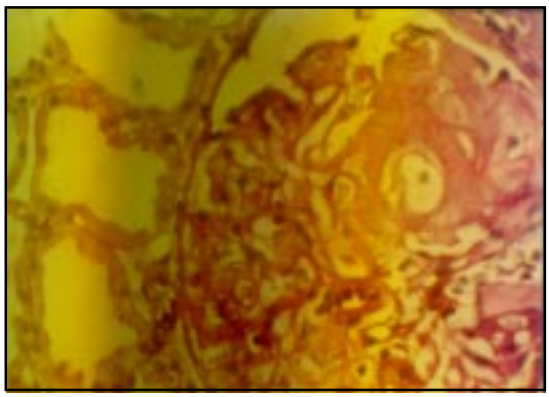

Figure 3. Amyloid in PAS stain shows pale pink homogenous structure in GBM and mesangium while normal GBM and tubular basement membrane have taken magenta pink colour of PAS (PAS ´ 500)

Table II
Associated Diseases in Renal Amyloidosis

Diseases	Duration of disease	No. (Total cases-11)	Percentage
TB lung	2.5 yrs to 10 yrs	3	27.27
Ankylosing Spondylitis + Cervical spondylosis	8 yrs	1	9.09
Chronic Ulcerative Colitis with Rheumatoid arthritis	4 yrs	1	9.09
Lepromatous leprosy	10 yrs	1	9.09
Frequent Jaundice	8 yrs	1	9.09
Carcinoma Caecum	2 months	1	9.09
Total		**8**	**72.72**

Table III
Biochemical Findings of Renal Amyloidosis

Tests	**No.**	**%**	**Range**	**Mean**
Blood Urea			In mg/dl	
Raised	2	22.22	240-325	282.5
Normal	7	77.77	16-35	27.18
S. Creatinine			In mg/dl	
Raised	2	22.22	8.1-4.5	11.3
Normal to borderline increased	7	77.77	0.7-1.2	1.22
24 hr. Urinary protein			In mg/dl	
Nephrotic range	6	85.71	4.87-12.3	7.63
Non nephrotic range	1	14.2	2.5	2.5

Table IV
Urinary Findings in Renal Amyloidosis

	Total Cases- 10	%
Proteinuria		
3 + to 4 +	9	90.90
2 +	1	10.00
Pus cells	8	80.00
RBC	2	20.00
Granular casts & Hyaline casts	9	90.00
Epithelial casts	2	20.00

Table V
Histological Changes in Renal Amyloidosis

Glomerular changes	**Total cases - 13**	**%**
Focal hyaline GBM thickening	11	84.61
Hyaline mesangial deposit	8	61.53
Hyaline deposit in AA	2	15.38
Hyaline deposit in EA	1	7.69
Glomerulomegaly	5	38.46
Fibril formation in hyaline deposit	6	46.15
Nodule in mesangium like NGS	2	15.38
Global glomerulosclerosis	4	30.76
Periglomerulitis & fibrosis	2	15.38
Mild mesangial cell proliferation	2	15.38

Table VI

Tubular Vascular and Interstitial changes in Amyloid Nephropathy

Tubular Changes	Total cases-13 N (%)	Blood Vessels	Total No. (%)
Patchy atrophy	2 (15.38)	Hyaline Thickened BV	
		a) Medium sized	9 (69.23)
		b) Capillary and arteriole	3 (23.07)
Thickened TBM	2 (15.38)	Hyaline Thickening	4 (30.76)
Focal Necrosis	6 (46.15)	of Bowmen's	
RBC cast	2 (15.38)	Capsule	
Thyroidisation of tubule	6 (46.14)	Interstitium	
Cellular cast	3 (23.07)	Patchy MNC	8 (61.53)
Vacuolar Degeneration	13 (100)	Focal Fibrosis	4 (30.76)
Hyaline Globules in PT	1 (7.69)	Granuloma	1 (7.69)
TBM Duplication	1 (7.69)		

Table VII

Special Stains Findings in Renal Amyloidosis

Special stain (Total No. cases - 12)	No.	%
AFOG		
a) Pale red area surrounded by dark blue membrane associated with	8	77.77
b) Red deposit in surrounding mesangium	2	16.66
c) Blue fibrils in amyloid area	4	33.33
PAS		
a) Negative	8	66.66
b) Positive (focal)	4	33.33
Congo Red		
a) Positive (red)	10	83.33
b) Positive (orange)	2	16.66
Methyl violet		
a) Negative	2	16.66
b) Positive (Rose pink)	10	83.33
Potassium permanganate treatment - not succeeded, section desquamated		

Table VIII

Polarizing Light Microscopy of Congo red Sections

Findings (Total cases - 10)	No. of cases	%
ι Focal peripheral, mesangium, blood vessel, green + yellow + red birefringence	10	100
ι Diffuse birefringence of whole amyloid	Nil	0
ι All LCDD (5) birefringence	0	0

Discussions

In the present study we reported 13 cases of renal amyloidosis in 5 years period with an incidence of 2% of total renal biopsy. Frequency of renal amyloidosis has been variable in different series. In the west, amyloidosis is an uncommon disease. Liu *et al* (9) from China reported only 14 cases of renal amyloidosis in 25 years period. In their series 64% patients were above the age of 50 years and primary amyloidosis was more common (79%). Study carried out from Kuala Lumpur Malaysia (10) reported 18 cases of renal amyloidosis. In their series age was between 25 to 64 years with mean age of 46 years with male predominance with a male female ratio of 2.6:1 like China. They found predominance of primary amyloidosis (10 out of 18 cases) and the diagnosis was based upon potassium permanganate treatment before Congo red staining. Similarly study carried out at Nephrology and Immunology Institute of Sarajevo found only 15 cases of renal amyloidosis in 15 years. They noted 82% cases of primary amyloidosis after immunohistochemistry and potassium permanganate treatment (11).

In India, incidence of renal amyloidosis in certain parts is high. Study carried out at Nair Hospital, Bombay which included (n=13) both autopsy and biopsy specimen (11) found 75 cases of renal amyloidosis in a period of 20 years which is higher than our incidence (13 cases in 5 years), which may be due to autopsy cases also. Like our study, their series also had 82.66% cases of secondary amyloidosis. High incidence of primary amyloidosis in western countries might be due to use of immunohistochemisty and histochemical staining while in our country it is purely based on clinical details. Now there are reports which suggest that clinical features are not sufficient to diagnose primary amyloidosis (12). Secondly, ours' being a tropical country, tuberculosis and leprosy form an important cause of amyloidosis. About 90% patient had nephrotic presentation while in other series it was seen in only 52% to 53.33% (9, 12).

History of associated diseases was found in 72.7% cases. One third of patients were known cases of tuberculosis for 5 to 15 years duration with incomplete treatment. In our country tuberculosis is the commonest cause. Shah *et al* (11) reported incidence of tuberculosis of various organs in 79% cases.

Similarly reports from western countries are also available regarding association of tuberculosis and renal

amyloidosis (13). Other chronic inflammatory conditions which are frequently involved in pathogenesis of amyloidosis include inflammatory bowel disease (IBD), rheumatoid arthritis and ankylosing spondylitis. We noted one case, each of ankylosing spondylitis and chronic ulcerative colitis. All were suffering from the disease for more than 5 years. Amyloidosis has been reported in 1 to 29% patients of IBD in adults (14). Contrary to our country where rheumatoid arthritis is a rare cause of amyloidosis, in western countries it is the commonest cause. In one series (15). 29 cases of rheumatoid arthritis associated amyloidosis were reported in a period of 11 years and 83% of these cases had survival of 42±8 months. In another series, researchers found that 10.5% of total rheumatoid arthritis patients had amyloidosis which affected mostly the second part of the duodenum (88.6%), duodenal cap (76.5%), and gastric antrum (68.9%). Mean duration of rheumatoid arthritis was 15.4 years and the presentation was mostly gastrointestinal (58.9% years), renal (58.9%) and cardiac symptoms (39.5% years) with 4 years survival of 57.8% (16). Similarly, reports of renal amyloidosis in ankylosing spondylitis and lepromatous leprosy are available in both Indian and Western literature (17, 18).Our histopathological findings were not much different from others. Routine stains like PAS, AFOG were not taken up by amyloid; Congo red stain was the only stain positive in all cases. Polarizing microscopy differentiated amyloidosis from light chain diseases but potassium permanganate treatment was not found to be suitable in differentiating primary from secondary amyloidosis. Almost similar findings were reported by other workers also (8).

Conclusion

Hence our study concludes that renal amyloidosis is common in our area. Chronic infection is the main causative factor hence it should be treated to prevent dreadful complications of renal amyloidosis. Secondly, in diagnosis of amyloidosis polarized microscopy of Congo red section should be done to confirm amyloid & serum electrophoresis and urine Bence Jone Protein to confirm primary amyloidosis in patients above 40 years of age because chronic infection may cause AA amyloidosis and AL amyloidosis also by chronic stimulation of B cells.

Refeences

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