

IgA NEPHROPATHY IN PAKISTAN

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Abstract

A light, electron and immunofluorescence microscopy study was performed on 102 consecutive patients on whom suitable percutaneous renal biopsies were obtained. In this selected group of patients primary IgA glomerulonephritis was diagnosed in 6 (5.9%) cases. On light microscopy the glomerular lesions were predominantly focal (WHO class III) and diffuse mesangial proliferative glomerulonephritis (Class IV). The mesangial deposits showed high association with IgM deposits and presence of early complement components (C1 q, C4) indicative of both classical and alternative pathways of C3 activation in our patients. The high incidence of nephrotic syndrome with microhaematuria (5 cases) is due to patient selection when compared to other studies. This study shows the existence of IgA nephropathy in Pakistan and larger number of cases need of this disease and its clinical manifestations and to be investigated to determine the true prevalence importance in Pakistan (JPMA 40 31, 1990).

INTRODUCTION

IgA nephropathy was first described by Jean Berger in 1968¹⁻². Following this report numerous papers came from France, USA, Japan and Singapore³⁻¹⁰. However, significant work which supported Berger's disease in the early years of its discovery and which highlighted the magnitude of the disease in the Asian-Pacific region came from Singapore¹⁰. In Pakistan, IgA nephropathy has not been documented so far. We describe here the pathological findings and the clinical features of this disease entity.

MATERIAL AND METHODS

Patients Selection

The study was conducted on 102 consecutive Patients in whom suitable renal biopsy tissues were obtained by the Department of Nephrology, Jinnah Postgraduate Medical Centre, from January 1988 to August 1988. There were 73 patients with primary and 29 with secondary glomerulonephritis. All the patients were evaluated clinically and hospitalized for percutaneous needle biopsies. The biopsy tissues were sliced into three fragments; for light, immunofluorescence and electron microscopy.

Light Microscopy

The renal biopsy specimens were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin and sectioned at 2 U thickness. The sections were stained with haematoxylin and eosin (H&E), periodic acid-schiff (PAS), periodic acid silver-methenamine (PASM) and Masson's trichrome. Biopsies containing at least 5 glomeruli were considered adequate for evaluation.

Immunofluorescence Microscopy

The biopsy specimens were snap frozen in liquid nitrogen after covering with OCT cryoprotectant and stored at -25°C until they were transported in dry ice to the Department of Pathology, National University of Singapore where they were stored at -70°C. Cryostat sections were cut at a 4u and

prepared for direct immunofluorescence microscopy according to the procedure described by Sinniah et al¹¹. FITC antibody preparations used for the study included rabbit antisera to human IgG, IgA, IgD, IgE, IgM, IgA secretory piece, C3, C1q, C4, fibrinogen, HBs antigen, HBc antigen, Kappa and Lambda light chains and albumin (Hoechst-Behring Laboratories). Sections were examined using the Zeiss (universal) large fluorescence microscope with 200 W/4 Li super pressure mercury lamp and a III RS epi-fluorescence condenser. The primary (exciter) filter was blue-violet Zeiss 487707 with an excitation range of 450-490nm, the secondary (barrier) filter was orange filter LP520 Zeiss 467873 with a cut off at 520nm. For black and white photography, Kodak plus-x, 125 ASA, 35mm film was used. The intensity was graded subjectively from 0 to + 3; 0 being negative and + 3 maximum intensity (mild 1+; moderate 2+, and marked 3+) fluorescence.

Electron microscopy

Specimens were fixed in 4% glutaraldehyde at 4°C, postfixed in 1% Dalton's chrome-osmium fixative, processed and embedded in Araldite. Thin sections cut at 60-90 mu were stained with uranyl acetate and lead citrate. The sections were examined and photographed in a Philips 400T electron microscope.

Criteria for histological diagnosis

The definitions and classification of the glomerular lesions described in the present study were based on the uniform system of classification for glomerular diseases, proposed by the World Health Organization Committee¹².

RESULTS

Immunofluorescence Microscopy

Of the 102 cases of primary glomerulonephritis, eight (7.8%) were shown to have significant amounts of IgA deposits in the glomeruli, but 2 of them were excluded from the study as one had associated diabetes mellitus and the other eclampsia associated nephropathy. The remaining 6 (5.9%) cases classified as idiopathic or primary IgA nephropathy were analysed. The immunofluorescence findings summarizing the presence of immunoproteins in these 6 cases are shown in Table 1.

TABLE I. Combinations of immunoglobulins and complements in the Glomerular Mesangium.

Combinations	No. of Positive Cases	%
A	1	16.7
A-M-C ₃ -C _{1q} -Fibrin	2	33.2
A-M-C ₃ -C _{1q} -C ₄ -Fibrin	1	16.7
A-M-G-C ₃ -C _{1q} -C ₄	1	16.7
A-M-G-C _{1q} -C ₄	1	16.7
Total no. of cases	6	100.00

IgA mesangial deposits as the predominant or sole immunoglobulin were found in all the cases. In addition IgM was present in 5 (83.3%), IgG in 2 (33.3%), C1q in 4 (66.6%), C3 & C4 in 3 cases (50%) each, and fibrin in 2 (33.3%) of the cases. IgA of grade 1+ was found in 1(16.1%) patient as the sole

immunoglobulin. Five (83.3%) patients showed grades 2 + to 3+ IgA, whereas the intensity of fluorescence was not as marked with the other immunoglobulins, complements and fibrin. In 4 of 5 cases with IgM of grade 2+ to 3+ intensity and in the 2 cases with IgG of grade 2 + intensity, the fluorescence of the immunoglobulins did not exceed that of IgA. The constant site of IgA deposition was in the mesangium (Figure 1).



Figure 1. Immunofluorescence microscopy shows localisation of IgA (+3) mainly in the glomerular mesangium (Magnification x300).

In 3 (50%) cases, IgA and other proteins were also found in the paramesangium and in some of the peripheral capillary loops. IgA was found in extra-glomerular vessels in 3 (50%) cases, while IgM was seen in 2(33.3%) and IgG in 1(16.6%) of the cases. IgA tubular casts were seen in 1 case. The complement components, C3, C1q & C4, showed a distribution similar to that of IgA in the glomerulus. C3 was found in extraglomerular vessels in 2 (33.3%) cases, usually in the juxtaglomerular arterioles. C1q was present in the blood vessels in one case while C4 was not found in any patient. All the 6 cases were negative for IgA secretory piece, HBsAg and HBcAg.

Light Microscopy

The histological changes involving the glomeruli, tubules, interstitium and blood vessels are summarised in Table II.

Table II. Pattern of glomerular, tubulo-interstitial and vascular lesions in IgA Nephropathy.

Glomerular lesions (Class)		Superimposed glomerular lesions					Tubulointerstitial lesions grades			Vascular lesion grades			
		No. of Cases	Segmental Proliferation	Sclerosis	Adhesions	Segmental Crescents	Inflammatory Cells	Mild	Moderate	Severe	Mild	Moderate	Severe
I.	Minimal change	-	-	-	-	-	-	-	-	-	-	-	-
II.	Minor change 1 (16.6%)	-	-	-	-	-	-	-	-	-	-	-	-
III.	Focal glomerulonephritis 1 (16.6%)	-	-	-	-	-	1	-	1	-	-	-	1
	(i) Segmental Proliferation	-	-	-	-	-	-	-	-	-	-	-	-
	(ii) Segmental Sclerosis	(1)	-	-	-	-	(1)	-	(1)	-	-	-	(1)
IV.	a) Diffuse mesangial proliferation	-	-	-	-	-	-	-	-	-	-	-	-
	b) Diffuse mesangial proliferation with superimposed lesions	2 (33.3%)	-	2	1	-	-	1	-	-	1	1	-
	(i) Mild	(1)	-	(1)	(1)	-	-	-	-	-	1	-	-
	(ii) Moderate	(1)	-	(1)	-	-	-	(1)	-	-	-	-	-
	(iii) Severe	-	-	-	-	-	-	-	-	-	-	-	-
V.	Diffuse Sclerosing glomerulonephritis	2(33.3%)	2 (mild)	-	-	1	-	-	2	-	-	-	2
	Total	6	2	2	1	1	1	1	3	-	1	1	3

All the cases showed mesangial widening, best seen with PAS, Masson's trichrome and silver stains. There was a wide range of glomerular lesions, comprising of minor change, focal and/or segmental lesions, diffuse, mesangial cell proliferation and diffuse sclerosing glomerulonephritis. Minor change showed widening of mesangium and a maximum of upto 3 nuclei per mesangial area in the peripheral capillary loops. One (16.6%) case belonged to this category (Figure 2),

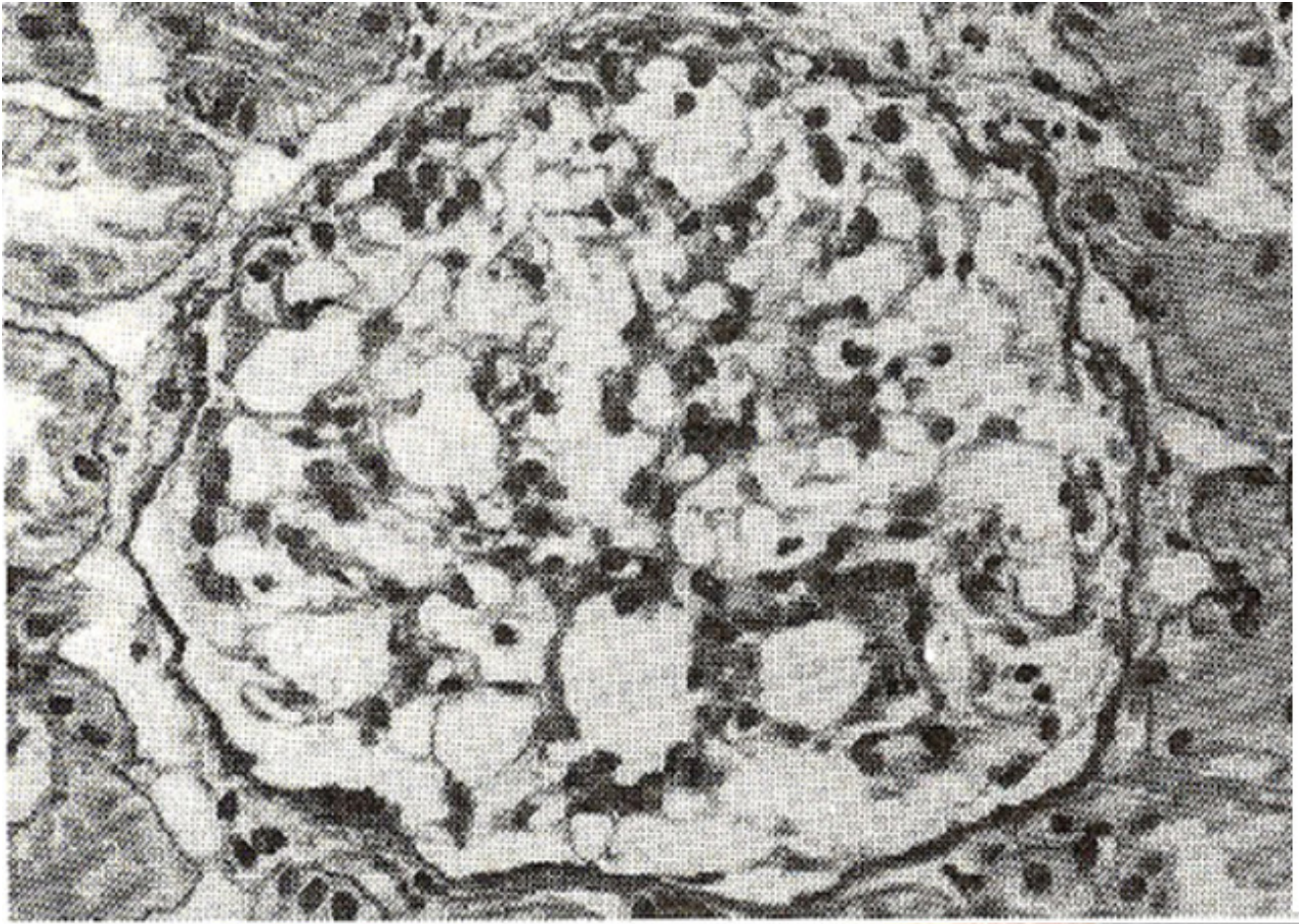


Figure 2. Minor change, with increase in mesangial matrix and up to a maximum of 3 mesangial cells per peripheral intercapillary mesangium (PAS, x400).

and one (16.6%) case had focal glomerulonephritis with segmental sclerosis (Figure 3),

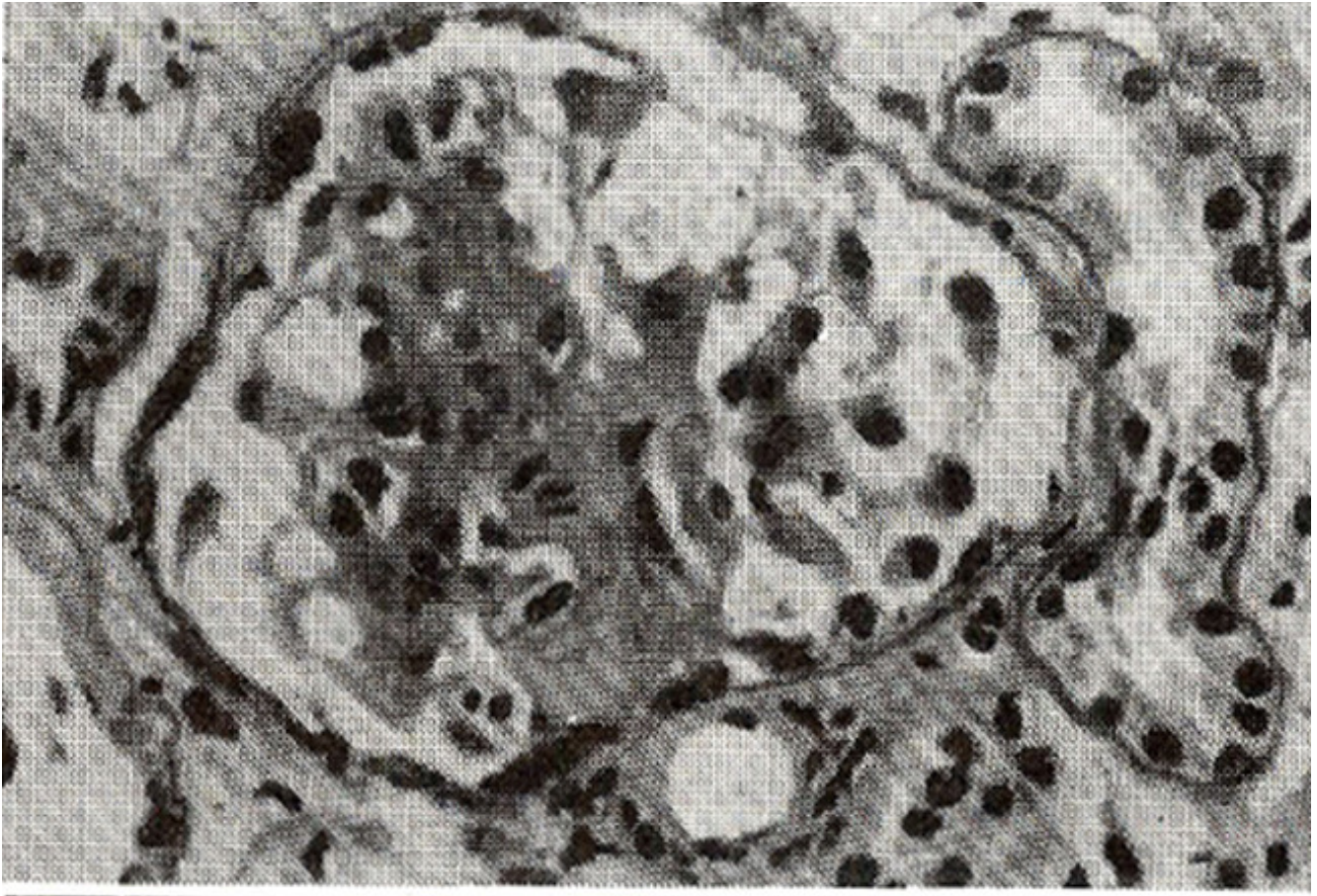


Figure 3. Focal | segmental glomerulonephritis, showing segmental perihilar sclerosis with hyalinosis (PAS, x650).

focal being defined as a lesion involving some but not all glomeruli (<80%). It also showed inflammatory cells comprising macrophages and polymorphs in some glomeruli. Diffuse proliferative glomerulonephritis involving all or nearly all (>80%) glomeruli was seen in 2 (333%) cases, with superimposed lesions of segmental sclerosis in both and adhesions to Bowman's capsule in one (Figure 4).

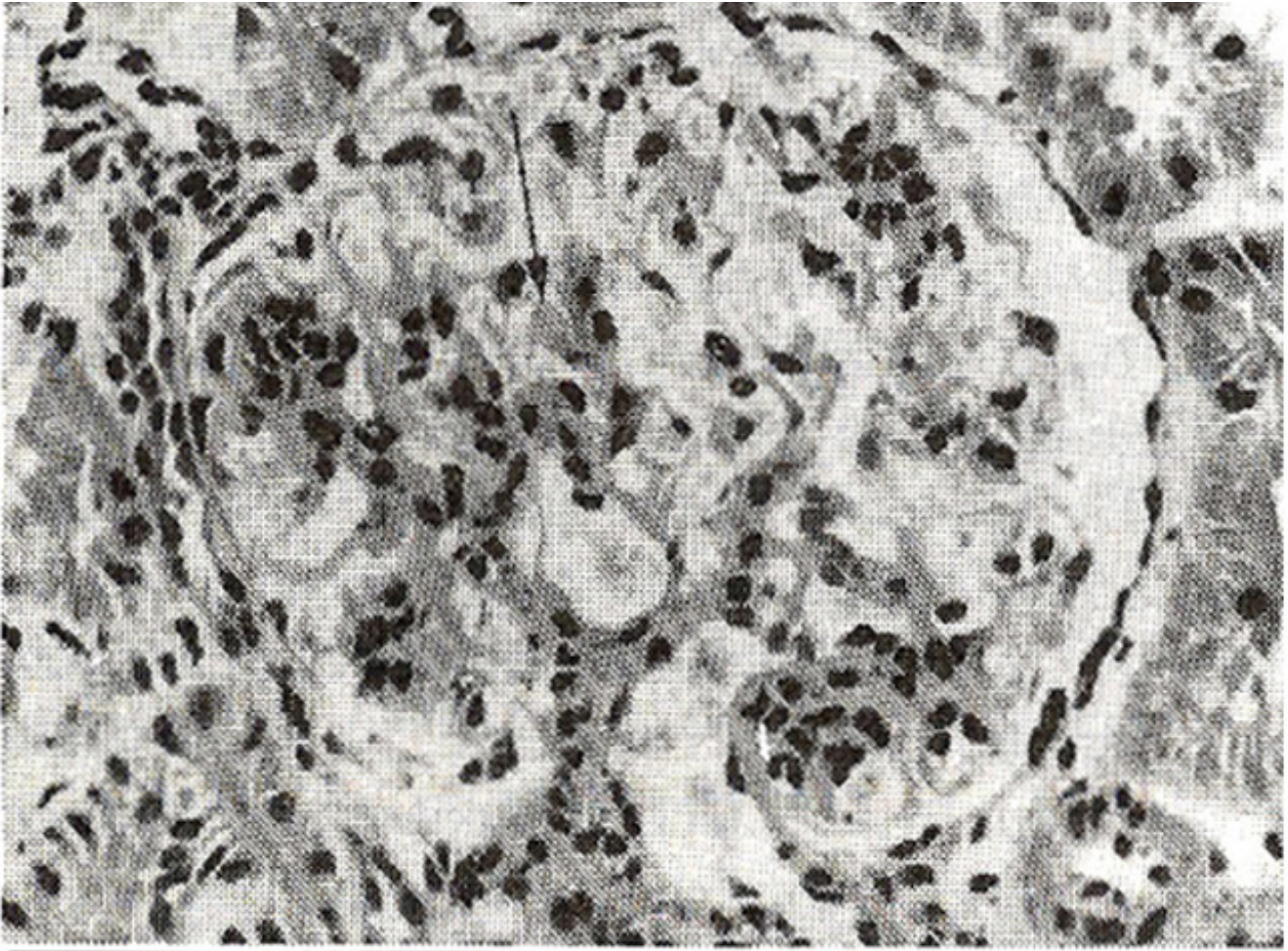


Figure 4. Diffuse mesangial proliferative glomerulonephritis, showing widening of mesangium and mesangial cell hyperplasia. A discrete globule (arrow) is seen (H&E, x400).

Mesangial deposits were detected in one (16.6%) patient as discrete droplets (Figure 4) identified with H & B, PAS, PASM and Masson's trichrome stains. Global sclerosis was frequently observed, and in 2 (33.3%) cases was extensive (>80%), and was classified as diffuse sclerosing glomerulonephritis. Superimposed lesion of segmental cellular crescent was seen in one case. Tubulointerstitial lesions of tubular necrosis, atrophy, interstitial fibrosis and inflammation were subjectively graded as mild, moderate and severe lesions. Four cases (66.6%) showed mild to moderate tubulointerstitial damage (TID), one (16.6%) mild, and 3 (50%) cases had moderate tubulointerstitial damage (Figure 5).

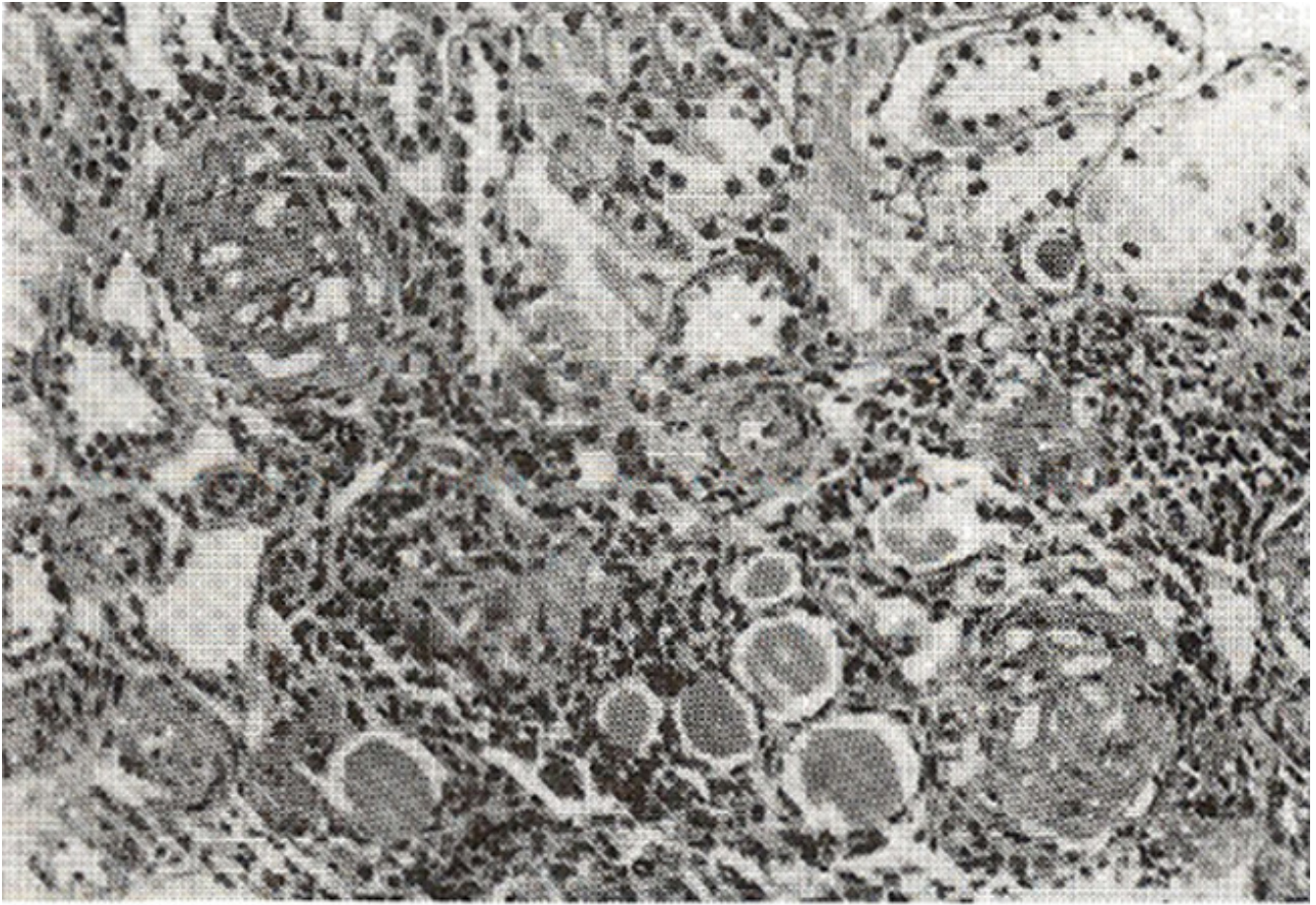


Figure 5. Tubulointerstitial and vascular lesions in IgA nephropathy. There is tubular necrosis and atrophy with lymphocytic infiltrates. Many tubules contain casts. There is mild to moderate degree of arteriolosclerosis. The glomeruli show global sclerosis (PAS, x350).

An attempt was made to correlate the glomerular pathology to the tubulointerstitial damage (TID). We observed that all cases with a moderate degree of TID had focal or diffuse sclerosing glomerulonephritis. One patient with mild TID belonged to the category of moderate diffuse mesangial proliferative glomerulonephritis with focal and segmental sclerosis as superimposed lesions. The two patients with minor change IgA nephropathy and diffuse mild mesangial proliferation with superimposed focal and segmental sclerosis did not show any TID. Vascular narrowing due to arteriolosclerosis was seen in 5 (83.4%) cases ranging from mild in one (16.6%), moderate in one (16.6%) to severe in 3(50%) cases (Figure 5). The patient with minor change IgA nephropathy showed no vasculopathy.

Electron Microscopy

Electron microscopy was performed in the six cases of idiopathic mesangial IgA nephritis. Electron dense deposits (EDD) were found in the glomerular mesangium in all cases (Figure 6).

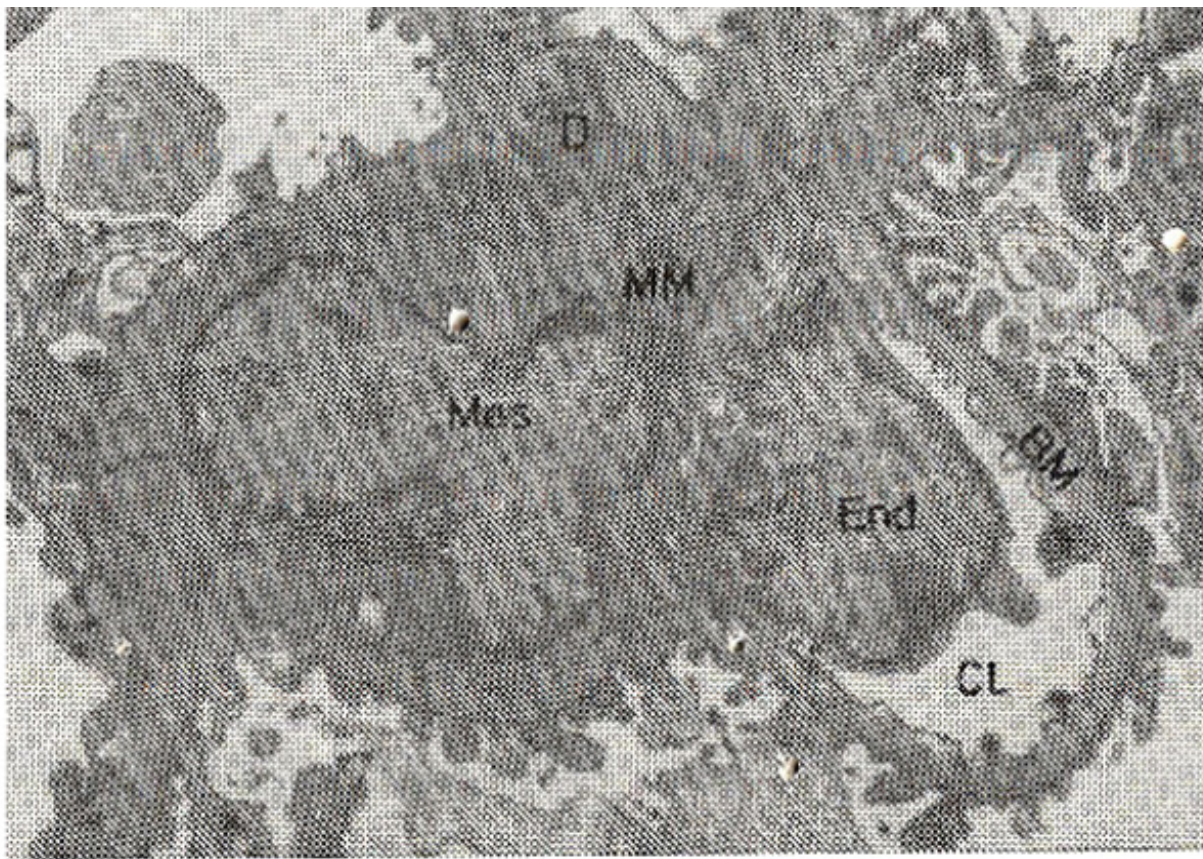


Figure 6. Electronmicrograph of part of a glomerulus showing electron dense deposits in the mesangium. Mes = mesangial cell; MM = mesangial matrix; End = endothelial cell; BM = Basement membrane; CL = capillary lumen; and D = dense deposits (Magnification x9,800).

Paramesangial extensions and deposits in the basement membranes of some peripheral capillary loops was seen in 2(33.3%) cases (Figure 7).

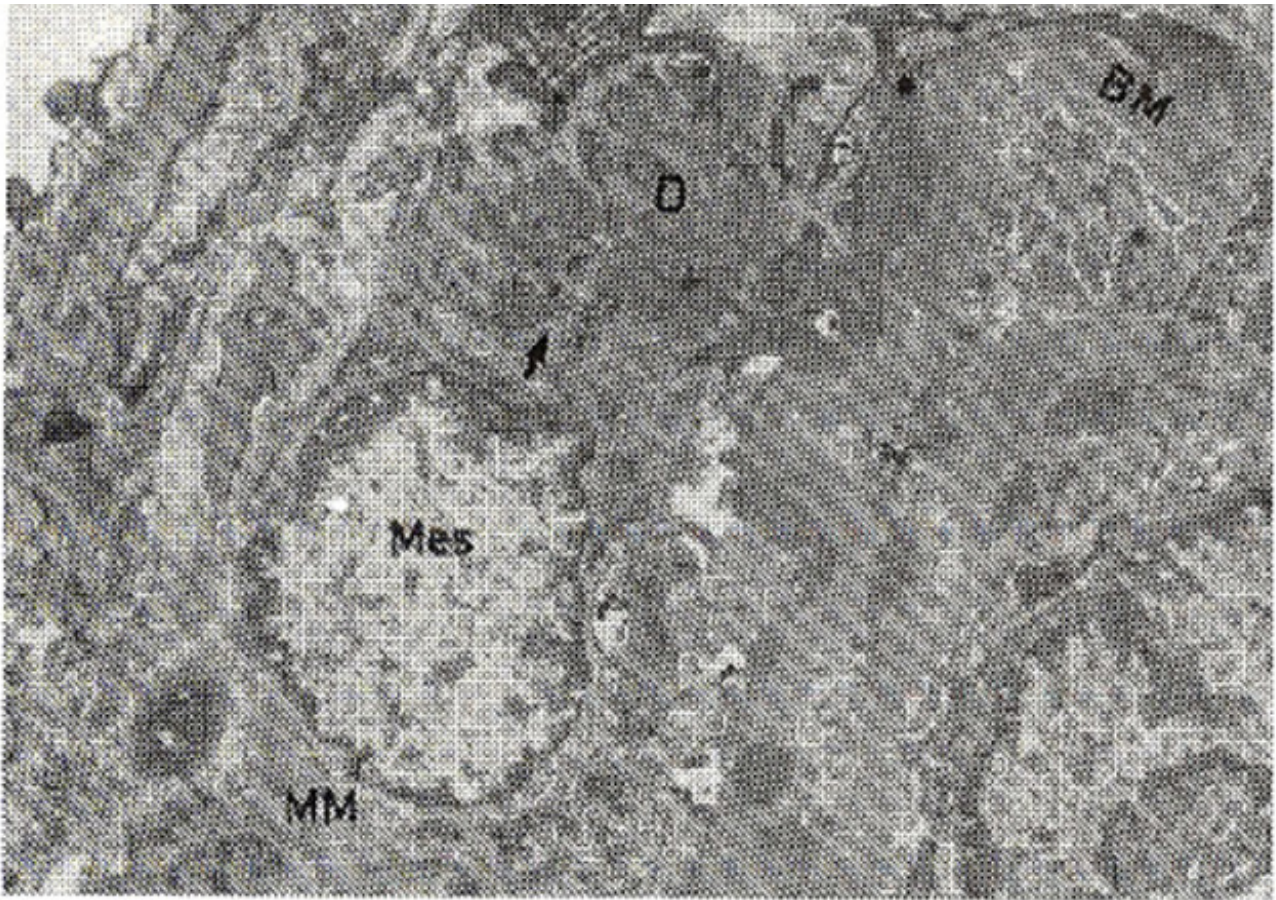


Figure 7. Electronmicrograph of part of a glomerulus showing electron dense deposits and collagen (arrow) in the mesangium. Peripheral extension of dense deposits are seen in the basement membrane. Mes = mesangial cell; MM = mesangial matrix; BM = basement membrane; Mes = mesangial cell; MM = mesangial matrix; BM = basement membrane; and D = dense deposits (Magnification x10,290).

Another constant feature was the increase in mesangial matrix with basement membrane like material. Mesangial cell changes, mainly in the form of increased rough endoplasmic reticulum and mitochondria was seen in cases with diffuse mesangial cell proliferation and focal or diffuse sclerosing glomerulonephritis. In cases with diffuse sclerosing glomerulonephritis additional changes of mesangial cell atrophy was seen. Collagen fibrils were seen in the mesangium in the cases of diffuse mesangial proliferation, and in sclerosing glomerulonephritis (Figure 7). All the cases showed areas of obliteration and broadening of foot processes with formation of microvilli. No inflammatory cell or platelet was seen in the capillary lumen. No double contour of capillary loops or tubuloreticular particles were seen.

Clinical features (Table III)

TABLE III. Clinical Features.

	No. of Cases
Total	6
Males	4
Females	2
Age, years	
0-19	3
20-49	1
> 50	2
Clinical Presentation	
Nephrotic syndrome \pm microhaematuria	5
Proteinuria/Haematuria	1
Haematuria RBC/HPF	
< 10	5
11-20	4
> 20	1
Creatinine clearance (< 30 ml/min)	2
Hypertension	4

The male to female ratio was 2:1, and the patients' ages ranged from 10 to 50 years. Five patients presented with nephrotic syndrome with accompanying microhaematuria in 4; and one presented with proteinuria/microhaematuria with 11-20 rbc per HPF. Creatinine clearance of <30ml/min was seen in two cases of diffuse sclerosing glomerulonephritis. Four cases also had hypertension at the time of presentation. An attempt was made to correlate glomerular lesions with clinical presentations. All the patients who presented with nephrotic syndrome had severe glomerular pathology, with diffuse mesangial proliferation, with superimposed segmental sclerosis, focal or diffuse sclerosing glomerulonephritis.

DISCUSSION

IgA nephropathy has been recognised as a distinct form of primary glomerular disease. The diagnosis is determined solely by immunofluorescence microscopy. The presence of IgA as the predominant immunoglobulin in the mesangium in patient with no systemic disease differentiates it from other diseases in which glomerular glomerular deposition of IgA occurs, as in systemic lupus erythematosus¹³, Henoch-Schonlein Purpura¹⁴ and alcoholic liver

cirrhosis¹⁵⁻¹⁶. In France and Italy IgA nephropathy is found in 20-25% of the adult patients with idiopathic glomerulonephritis^{4,17}. A higher incidence of 33.7% was reported from Singapore¹⁸, and between 35-40% from Japan¹⁹⁻²⁰. However, lower incidence ranging from 1.5 to 10% have been published from Britain, the United States and Canada^{6,8,21-22}. In our series the primary IgA nephropathy incidence of 5.9% is quite low compared to other Asian Pacific countries. A part from geographical variation, it may be related in part to the criteria of patient inclusion or selection for renal biopsy. However, larger number of cases have to be analysed to determine the true prevalence of this disease in Pakistan. The majority of our patients were young, with a predominance of males as reported by others⁴⁻⁸. We found a higher incidence of nephrotic syndrome than other workers^{18,21}, which could partly be due to the selection of patients for biopsy, and also patients coming late for treatment²³. The initial reports termed the lesion IgA-IgG mesangial nephropathy^{2,4-5,7,9,17,19}. An IgA-IgG combination was seen in approximately 50% of the cases^{18,22}. We observed an IgA-IgM combination was seen in 83.3% of the cases studied. The frequency of associated IgM is the highest report in the world literature. IgM was found in 60-70% of cases by many workers from North America (USA)^{8,24} and from Northern Europe (Holland)^{25,26}, whereas in all reports from Southern Europe (France, Italy and Spain), from Asian (Japan and Singapore), and from Australia the prevalence is lower²⁷. This discrepancy probably reflects a difference in the immunological response²⁷, possibly due to geographical differences related to environmental and/or genetic susceptibility factors^{18,28}. Another distinctive feature is the high frequency of early complement components of the classical pathway of C3 activation. The high frequency of C1q (83.3%) and C4 (50%), with grade 3+ staining in one case each is also the highest frequency reported so far²⁹. This suggests that along with the activation of complement C3 through the alternate pathway by aggregated IgA, the classical pathway is activated through IgG and IgM, in IgA nephropathy as shown by other workers^{30,31}. Electron microscopic changes of mesangial deposits with peripheral extensions, increased mesangial matrix and mesangial cell organelles, and formation of collagen fibrils with cellular atrophy have already been described in greater detail^{32,33}. The tubulointerstitial damage and arteriosclerosis with glomerular damage represent greater degree of damage with poor prognosis. This paper shows that IgA nephropathy exists in Pakistan, and more work needs to be done to determine the extent of the disease, the clinical presentations, and the possible causes of the disease(s).

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