Diagnosis of IgA Nephropathy by Immunohistochemical Methods

A Preliminary Study

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Summary

Ten patients who presented with haematuria, mild proteinuria or both were selected for this study. The age ranged from 12 years to 44 years and both sexes were included. Immunohistochemical stains revealed IgA immune-complexes in the mesangium in 4 patients. One had traces of IgM in addition to IgA. C_3 was present in all 4 patients along with IgA.

The present results show that the technique of immunohistochemistry could be used on formalin fixed paraffin embedded sections in the detection of IgA nephropathy.

Introduction

Mesangial IgA disease was first described by Berger in 1968[1] and in many countries it is now recognised as the commonest form of primary glomerulonephritis[2].

In this glomerulopathy immunological studies demostrate that IgA and CA_3 are found predominantly in the mesangial region together with smaller amounts of IgM and sometimes IgG and fibrinogen. In some cases the deposits extend around the capillary loops.

The incidence of IgA nephropathy is commoner in Southeast Asian countries than European countries. A higher incidence of 35-40% have been reported from Japan[3]. Lower incidences ranging from 4-10% have been reported from Britain, the United States and Canada[4,5]. It is primarily a disease of young men with a peak age of 15-30 years. The pathogenesis is not understood. The light microscopic changes vary from minimal change to crescentic glomerulonephritis. The present study was done to identify IgA nephropathy in a selected number of patients by using the technique of immunohistochemistry.

In addition this was the first time that immunological studies were performed on renal tissue in Sri Lanka.

Materials and methods

The study was conducted on a selected number of patients undergoing renal biopsy from January 1990 to October 1990, at the Professorial Unit of Clinical Medicine, Colombo. None of the patients had signs of systemic Lupus Erythematosus or Henoch Schonlein purpura. A full clinical examination with blood pressure readings and laboratory investigations for renal function was done. The clinical findings of our patients are shown in table I. A surgical biopsy of morphologically normal kidney was obtained from a patient who had a nephrectomy for a renal adenocarcinoma, as a control.

Light microscopy

Two cores of renal tissue were obtained from each patient. One was fixed in 10% formal saline and the other was fixed in Duboscq-Brazil solution. They were embedded in paraffin and 6mμ thick sections were stained with Haematoxylin and eosin, PAS and Silver methanamine.

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Table I

Clinical and Renal Functional States of the Patients with IgA Nephropathy

No.	*Age*	*Sex*	*Duration of Symptom*	*Protein Uria*	*Haemat-uria*	*Oede-ma*	*Renal Function*	*BP*
1	23	F	6/12	+	++	-	N	N
2	12	F	3/52	+		-	N	N
3	16	M	2/12	+		-	N	N
4	22	M	1/12	+		-	N	N
5	34	F	3/52	+	+	-	N	N
6	44	M	6/52	+		-	N	N
7	20	M	2/52	++	+	Mild Ankle	N	N
8	28	M	3/52	+	-	-	N	N
9	29	M	4/52	+	+	-	N	N
10	36	M	5/12	++	+	-	N	N

Immunohistochemical studies

Formalin fixed paraffin embedded sections were used and Avidine Biotin Complex method was performed. The primary rabbit antisera were obtained from Hoechst Ceylon Ltd. Biotinilated swine antirabbit sera and ABC complex were obtained from Dakopatts. We confirmed the reagent activity with known positive and negative biopsies as controls. A tonsil containing IgA +ve plasma cells was used as the positive control. A normal kidney was used as the negative control. The biopsies were trypsinized to get rid of blocking proteins. Endogenous peroxidase activity was inhibited by adding Hydrogen peroxide. All tissues were incubated with rabbit antisera to human IgG, IgM, IgA CA_3 CA_{1q} and Fibrinogen. The biotinilated swine antirabbit sera was added. Horseraddish peroxidase conjugated ABC complex was added and then the substrate 3-3 diaminobenzidine tetrahydrochloride (DAP)was added. The presence of Immunoglobulins was indicated by a brown staining.

Results: Light microscopy

The glomerular, tubular, interstitial and vascular changes in the ten cases are summarized in Table II. Four patients had only minimal change glomerulonephritis. Two patients had largely minimal change with only focal mesangial hypercellularity. Other 4 patients had diffuse mesangial proliferative glomerulonephritis with increased amount of mesangial matrix. There were no epithelial cresents, glomerulosclerosis, vascular changes and tubulointerstitial disease in any of these biopsies.

Immunohistochemistry

The results were recorded by ordinary light microscope. Presence of IgA complexes were indicated by granular brown staining of the mesangial martrix. IgA immune complexes were present in 4 of our cases, in the mesangium. There was no staining around the capillaries. One case had traces of IgM in

Table II

Pathological Findings in the Patients with IgA Nephropathy

Patient No.	*Diagnosis*	*Increased Mes. Cells*	*Increased Matrix*	*Mesangial Deposits*
1	Minimal Change	-	-	+
2	Minimal with Focal Mesangial Prolife-ration	+ Focal	+ Focal	+
3	Mesangial Prolife-rative	++	+	-
4	Minimal	-	-	-
5	Mesangial Prolife-rative	++	++	+
6	Mesangial Prolife-rative	+	+	-
7	Minimal	-	-	-
8	Minimal	-	-	-
9	Mesangial Prolife-rative	++	++	+
10	Minimal with Focal Mesangial Hypercel-lularity	+ Focal	-	-

addition to IgA. C3 was also present in 4 patients who had IgA. IgG and fibrinogen were not seen in any of our cases. The positive control revealed IgA containing plasma cells.

Discussion

The diagnosis of mesangial IgA nephropathy is determined solely by immunological methods. Until recently majority of immunologically mediated renal diseases were diagnosed by the classical immunofluorescence method. But it has several disadvantages like instability, difficulty in quantitation, expensive equipment for its examination and recording results. But now immunohistochemistry has been performed in various centres all over the world as an alternative method in the diagnosis of renal disease. IgA nephropathy is primarily a disease of young men, with a peak age of 15 to 30 years. The pathogenesis is not understood. The occasional development of IgA nephropathy in patients who have chronic

inflammatory lesions of IgA containing mucosal surfaces such as gluten enteropathy, Crohn's disease and chronic bronchitis suggests an increased entry of exogenous mucosal antigens (those of viruses or bacteria or food antigens) into the general circulation where they complex with IgA.

Although it was once felt that this was a benign disease it is now becoming clear that up to 20% of these patients develop end-stage renal failure. The serum IgA was not tested in our study. The disease has a wide range of clinical presentation. The common manifestations are microscopic and gross haematuria, proteinuria, acute nephritis and nephrotic syndrome. The less common presentations are hypertension and rapidly progressive glomerulonephritis. IgA nephropathy is a mesangial proliferative lesion, meaning that mesangial cell proliferation is the sole or predominant abnormality. But the histological pattern could be so variable that focal segmental lesions, epithelial crescents and glomerulosclerosis, could be seen in longstanding cases. Adhesions and tubulointerstitial damage had been reported in larger studies. In our 4 cases with +ve IgA disease 3 were females and one was male. The duration of the symptoms were not more than 6 months. The glomerular changes in our cases were minimal and mesangial proliferation only, probably due to the short duration of the illness.

This is the most common type of adult primary glomerulonephritis in many parts of the world accounting for over 20% of cases in France, Italy, Australia, Japan and Singapore[2]. In Sri Lanka the incidence had not been documented and by this technique of immunohistochemistry we could make a definite diagnosis of IgA nephropathy.

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REFERENCES

1. Berger J.& Hinglais N. Les depots inter capillaries d IgA-IgG., *Journal of Urology and Nephrology,* 1968, 74, 694-695.
2. Sinniah R. Javier A.R. & Ka G. The pathology of mesangial IgA nephritis with clinical correlation, *Histopathology,* 1981, 5, 469-490.
3. Sakai O. Kitajima T. Kawamura K. & Ueda Y. Clinicopathological studies on IgA glomerulonephritis, In. Glomerulonephritis, Proceedings of the International symposium on glomerulonephritis, (1977) 167-180.
4. Lowance D.C. Mullins J.D. and Mcphaul J.J.Jr. Immunoglobulin A (IgA) associated glomerulonephritis. *Kidney International,* 1973, 3, 167-176.
5. McCoy R.C. Abramowsky C.R. & Tishev C.C. IgA nephropathy, *American Journal of Pathology,* 1974, 76, 123-144.