ORIGINAL ARTICLE

Morphological Patterns of IgA Nephropathy with aid of iNOS

ATIKA MASOOD[1], MUNAZZA AZAM[2], ALIA WAHEED[3]

ABSTRACT

Background: The most common form of primary glomerulonephritis is primary or idiopathic IgA nephropathy. The mainstay of diagnosing IgA nephropathy remains immunofluorescence microscopy which shows predominantly IgA deposits and C3 scapillary walls. Induction of iNOS occurs as an initial rapid response to immune insult in glomerulonephritis (GN).

Methods: One hundred and thirty two consecutive patients of nephrotic and nephritic syndrome, both children and adult, were included in the study. After baseline investigations and serum IgA level, 33 patients having clinical suspicion of IgA nephropathy and 1 patient of Henoch Schonleinpurpura nephritis were admitted and renal biopsies were taken by well trained nephrologists after consent from the patients and/ or parents of the patient in care of a child. These cases were selected from Sheikh Zayed Hospital, Children Hospital, Services Hospital, Fatima Memorial Hospital and Jinnah Hospital Lahore.

Results: Among these 34 renal biopsies, 23(67.65%) were males and 11 (32.35%) were females. The minimum age at biopsy was 2 years and maximum was 73 years, mean±S.D of age was 28.18±19.62.Among the 34 patients microscopical haematuria was present in 17 (50%) and macroscopic haematuria in 10 (29.4%). Duration for haematuria was minimum 1 month and maximum 72 months with mean±S.D of 10.0±15.63.Among the 34 patients 32 were detected to have proteinuria. Sixteen (47.1%) had < 2g/ 24hrs while 16(47.1%) had > 2g/ 24hrs proteinuria. Duration of proteinuria was minimum 1 month and maximum 24 months with mean±S.D of 6.59±6.66.The minimum serum creatinine was 0.60 mg/dl and maximum serum creatinine was 12.80 mg/dl with a mean±S.D serum creatinine being 2.92±3.14 mg/dl. Serum IgA level was performed in all the 34 patients out of which 20 (58.82%) showed raised level while 14(41.17%) cases showed normal IgA levels. Among 34 clinically suspected of IgA nephropathy, 17 cases turned out to be of IgA nephropathy after morphological and IF studies.

Conclusion: Diagnosis of IgA nephropathy cannot be made clinically as it has not proven a reliable method so renal biopsy in addition to the H&E and histochemistry should be examined using immunofluorescence that is mandatory for the correct diagnosis of IgA nephropathy iNOS expression can differentiate between proliferative and non proliferative forms of IgA nephropathy at a very early stage, hence very helpful in making a correct diagnosis.

Keywords: IgA nephropathy, Glomerulonephritis, iNOS

INTRODUCTION

It is almost forty two years since Jean Berger was able to describe IgA nephropathy as recent group of primary GN. Berger discovered IgA nephropathy using immunohisto-chemistry (IH) and renal biopsy examination[1]. Now Berger disease is considered as a syndrome having uniform morphology but clinical features show great diversity hence variable prognosis[2]. Woo et al, showed that IgA nephropathy has renal survival after five years as 89%, after ten years it is 81%, and after twenty years it is 65%. The deterioration is a slow and progressive process, taking an average 7.7 years[3]. IgA nephropathy prevalence shows distinct geographic variation as it is highest in Singapore, Hong Kong, Japan, Australia, Finland and southern Europe where it accounts for 20% -40% of cases of primary GN. While in US, UK and Canada the prevalence is very low[4].

The most important pathogenic factor in primary or autoimmune IgA nephropathy is the alteration in IgA biology.In IgA nephropathy, the IgA1 molecule contains reduced glycosylation of O-linked glycan in hinge region resulting in the formation of terminal GalNac residues[5]. IgA1 level in the plasma is elevated in 50% of the patients of IgA nephropathy[6].

[1]Associate Professor of pathology AMDC,
[3]Assistant Professor of pathology AMDC, Contacy number 03234624000,alphahsan@yahoo.com.
Correspondence to Dr. Atika Masood Email: dratika@hotmail.com, Cell: 03349071762

Light microscopy shows histological variability in this disease, ranging from minimal change to diffuse proliferative glomerulonephritis (GN) to crescenteric GN. The immunofluorescent microscopical study of IgA nephropathy shows mesangial deposits of IgA-C3 predominantly with IgG or IgM. The deposition of immune complexes is global and diffuse intercapillary irrespective of light microscopic lesions, whether or not they are focal and segmental[7]

The increased interstitial expression of iNOS is associated with clinical indicators of poor prognosis in IgAN. Macrophages have the potential to cause renal injury by inducing production of reactive oxygen species, nitric oxide and various pro-inflammatory cytokines. Nitric oxide is synthesized from L-arginine by nitric oxide synthase via the p38 mitogen-activated protein kinase (MAPK) signaling pathway[8]. iNOS expression by interstitial macrophages is detected in IgA nephropathy. The number of iNOS positive cells correlate with tubulointerstitial fibrosis and decline in renal functions[9]. iNOS positive cells are seen in the glomeruli of proliferated forms of IgAN and lupus nephritis but not in non proliferative form of GN.

MATERIALS & METHODS

This study was conducted in the Department of Morbid Anatomy and Histopathology, at University of Health Sciences, Lahore. A detailed history, socio- demographic information physical and systemic examinations were

performed. Serum IgA level, serum creatinine, urinalysis results, ASO titre, ANF, anti-DNA, serum complement levels (C3 and C4), 24 hours urinary protein excretion, creatinine clearance were carried out wherever it was possible.One hundred and thirty two consecutive patients of nephrotic and nephritic syndrome, both children and adult, were included in the study.After baseline investigations and serum IgA level, 33 patients having clinical suspicion of IgA nephropathy and 1 patient of Henoch Schonleinpurpura nephritis were admitted and renal biopsies were taken by well trained nephrologists after consent from the patients and/ or parents of the patient in care of a child.Samples were collected from the department of Nephrology Institute of Child Health & Children Hospital, Services institute of medical sciences, Fatima memorial Hospital, Jinnah Hospital and Sheikh Zayed Hospital, Lahore. Two cores of renal biopsies were obtained from each patient under real-time ultrasound guidance to localize the kidney, using a needle biopsy gun.The core for light microscopy was sent in the 10% formol saline and a sample for immunofluorescence study was transported in the parafilm placed in petri dish on ice in insulated box. IF staining was done with antibodies specific for the heavy chains of IgG (sheep polyclonal antibodySH-1921-R2), IgA(sheep polyclonal antibodySH-1920-R2), IgM (rabbit polyclonal antibodyRB-1922-R2), C3(sheep polyclonal antibodySH-1923-R2), C1q(rabbit polyclonal antibodyRB-1926-R2) and fibrinogen(rabbit polyclonal antibodyRB-1924-R2). The biopsies were stained with H&E, JMS, Masson trichrome, PAS and IHC staining with ANTI iNOS antibody (ab. 49999).Immunostaining was scored based on the intensity of staining and the percentage of cells that stained positively. Whenever there was a disagreement between the two persons viewing, the slides were reviewed by the Professor and consensus was reached. Staining scores were calculated by multiplying the percentage of positive inflammatory cells per section by the staining intensity.The intensity score represented the estimated staining intensity of the cytoplasm of the inflammatory cells.

Intensity of staining

0, No Staining
1+ Weak; Focal
2+ Moderate; Focal
3+ Strong ; Diffuse

Extent of staining

1+ (1%-25%)
2+ (26%-50%)
3+ (51%-75%)
4+ (>75% of stained glomerular tufts or tubules)

RESULTS

Among these 34 renal biopsies, 23 (67.65%) were males and 11(32.35%) were females. The minimum age at biopsy was 2 years and maximum was 73 years, mean±S.D of age was 28.18±19.62.Among the 34 patients microscopical haematuria was present in 17 (50%) and macroscopic haematuria in 10(29.4%). Duration for haematuria was minimum 1 month and maximum 72 months with mean±S.D of 10.0±15.63.Among the 34 patients 32 were detected to have proteinuria. Sixteen (47.1%) had < 2g/ 24hrs while 16(47.1%) had > 2g/ 24hrs proteinuria. Duration of proteinuria was minimum 1 month and maximum 24 months with mean± S.D of 6.59±6.66.In all the 34 patients, the minimum serum creatinine was 0.60 mg/dl and maximum serum creatinine was 12.80mg/dl with a mean± S.D serum creatinine being 2.92±3.14 mg/dl. The minimum serum bilirubin in the 34 renal biopsies was 0.24 mg/dl and maximum serum bilirubin was 1.90mg/dl with a mean±S.D serum bilirubin being 0.65±0.42mg/dl. The minimum serum albumin was 1. 0 g/dl and maximum was 5.10 g/dl with a mean±S.D of serum albumin being 3.98±0.84g/dl.The urinary proteins ++++ were seen in 2 (5.9%) patients, +++ in 13(38.2%) cases, ++ in 14 (41.2%) and + in 4(11.8%) patients. The values for urinary proteins as graded above are as follows:

+ ---------- 30 – 100 mg
++ --------- 100 – 150mg
+++ ------- upto 2g
++++ ----- more than 2g

Serum IgA level was performed in all the 34 patients out of which 20 (58.82%) showed raised level while 14 (41.17%) cases showed normal IgA levels.

All the biopsies, in addition to the Haemotoxylin- eosin were stained with Periodic acid Schiff's reaction (PAS) to view the mesangial matrix ,potential expansion in matrix, mesangial cells, alterations in basement membrane and vessels, masson'strichrome to see the extent of fibrosis and Jones Methenamine silver stain for the detection of changes in glomerular basement membrane (GBM). The results were as follows: Among the 34 clinically suspected cases of IgA nephropathy, 17 (50%) cases turned out to be of IgAN after immunofluorescence study, In the study we have tested the relationship of serum creatinine of suspected patients of IgA Nephropathy and Expression of iNOS and it shows significant relationship.

We had tested the Hypothesis for Proliferative Pattern Of IgA Nephropathy And iNOS Expression and in the present study we have concluded that there is strong association between Proliferative pattern of IgA nephropathy and iNOS expression (p- value 0.000).

Table 1: Histopathological Diagnosis in 34 patients

Diagnosis	Frequency	%	Valid %	Cumulative%
Valid				
Minimal Change Disease	2	5.9	5.9	5.9
Focal Mesangial Proliferative Nephritis	9	26.5	26.5	32.4
Diffuse Mesangial Proliferative Nephritis	3	8.8	8.8	41.2
Focal Segmental Glomerulosclerosis	7	20.6	20.6	61.8
Membranoproliferative Glomerulonephritis	4	11.8	11.8	73.5
Membranous Glomerulopathy	2	5.9	5.9	79.4
Diffuse Proliferative Glomerulonephritis	1	2.9	2.9	82.4
End Stage Renal Disease	6	17.6	17.6	100.0
Total	34	100.0	100.0	

Table 2: Number of detected IgAN cases among 34 clinically suspected patients

Type of renal lesion	Number of IgAN Cases	Total no of cases
Minimal Change Disease	0	01
Focal Mesangial Proliferative Nephritis	5	09
Diffuse Mesangial Proliferative Nephritis	1	03
Focal Segmental Glomerulosclerosis	2	07
Membranoproliferative Glomerulonephritis	2	04
Membranous Glomerulopathy	1	02
Diffuse Proliferative Glomerulonephritis	1	01
End Stage Renal Disease	5	06
Total	17	34

Comparison of IgAN classes and intensity of staining with Anti iNOS antibody was done and results are as follows

Table 3: Histopathological diagnosis * intensity of staining with Anti iNOS antibodycross tabulation

Histological diagnosis		%	Valid %	Cumulative%
Minimal Change Disease	Negative	1	50.0	50.0
	+	1	50.0	50.0
	Total	2	100.0	100.0
Focal Mesangial Proliferative Nephritis	+	1	11.1	11.1
	++	5	55.6	55.6
	+++	3	33.3	33.3
	Total	9	100.0	100.0
Diffuse Mesangial Proliferative Nephritis	++	2	66.7	66.7
	+++	1	33.3	33.3
	Total	3	100.0	100.0
Focal Segmental Glomerulosclerosis	Negative	5	71.4	71.4
	+	1	14.3	14.3
	++	1	14.3	14.3
	Total	7	100.0	100.0
Membranoproliferative Glomerulonephritis	+	2	50.0	50.0
	++	1	25.0	25.0
	+++	1	25.0	25.0
	Total	4	100.0	100.0
Membranous Glomerulopathy	Negative	1	50.0	50.0
	+++	1	50.0	50.0
	Total	2	100.0	100.0
Diffuse Proliferative Glomerulonephritis End Stage Renal Disease	++	1	100.0	100.0
	+	2	33.3	33.3
	++	2	33.3	33.3
	+++	2	33.3	33.3
	Total	6	100.0	100.0

Table 4: Histopathological Diagnosis of IgAN * Intensity of Staining with Anti iNOSantibody Cross tabulation

Histopathological Diagnosis	IgAN Cases	Intensity of Staining with Anti iNOS			
		-VE	+	++	+++
Focal Mesangial Proliferative Nephritis	5	0	1	3	1
Diffuse Mesangial Proliferative Nephritis	1	0	0	0	1
Focal Segmental Glomerulosclerosis	2	2	0	0	0
Membranoproliferative Glomerulonephritis	2	0	1	1	0
Membranous Glomerulopathy	1	1	0	0	0
Diffuse Proliferative Glomerulonephritis	1	0	0	1	0
End Stage Renal Disease	5	0	1	2	2
Total	17	3	3	7	4

Table 5: Relationship of serum creatinineof suspected patients of IgA Nephropathy and Expression of iNOS by IHC

Clinical Parameters	P- Value
Serum Creatinine (mg/dl)	0.040

Level of significance: $\alpha = 0.05$

DISCUSSION

Glomerulonephritis (GN) encompass a diverse category of renal diseases with a broad spectrum of pathological outcomes. The most common form of primary GN in developed world is IgA nephropathy (IgAN) that it is also an important cause of end stage renal disease[10,11]. IgA nephropathy is described immunologically by the deposition of IgA immune complexes in the mesangial region in the setting of diverse clinical features, but mainly proteinuria and asymptomatic haematuria[1].

Diagnosis of IgA nephropathy usually begins with a clinical suspicion. We, in the present study also selected 34 renal biopsies of IgA among a total of 132 on clinical grounds including history, routine blood and urine analysis. The clinical suspicion is based on recurrent episodes of macroscopic haematuria or persistent microscopic haematuria with mild to moderate proteinuria.

Epidemiological studies around the world show that IgA nephropathy is universally distributed but with varied frequencies due to ancestral differences and policies regarding renal biopsy practices. The IgA nephropathy prevalence is highest in Hong Kong, Japan, Singapore, and Australia accounting 20 – 40% cases of primary glomerulonephritis and accounts as low as 2% in America, Canada and England[4]. In our study, among 132 cases of glomerulonephritis, 17 (12.87%) cases turned out to be of IgA nephritis and one case was of Henoch–Schonlein Purpra associated Nephritis (HSPN) and our results are in accordance with the previous studies reported from Pakistan[12] but in two studies reported from southern part of country the reported frequency was 2% and 5.9% however the immunofluorescence techniques were not applied in later ones[13,14] (Khan, 1988) (Khan, 1990). The study from the northern parts of Pakistan showed the prevalence of IgA nephropathy to be 7.9%[15]. In India the incidence of 8.9%[16] and 14.26% were reported[17].

The present study showed that the use of H&E, JMS, and trichrome stains revealed the key features of different histological classes of IgA nephropathy. H & E stains provide the first impression of the composition of the renal tissue and to analyse the glomerulus, PAS stain is most useful as it delineates in great detail the glomerular cells, mesangial matrix, potential expansion of matrix, changes in GBM and fibrinoid necrosis of glomerular tuft. JMS showed irregularities and thickening of the GBM as well as spikes in capillary loops and vascular basement membrane. Trichrome stains helps in evaluating the extent of fibrosis in glomerular or tubulointerstitial compartment. They gave almost a clear histological picture of the biopsy but IF remains the gold standard in the diagnosis of IgA nephropathy and to differentiate it from other nephropathies[18].

Light microscopy shows histological variability in IgA nephropathy, ranging from minimal change to diffuse proliferative glomerulonephritis (GN) to crescenteric GN. Mesangialhypercellularity is mainly focal segmental and sometimes it is accompanied by mesangial matrix expansion[19]. Necrotizing lesions like disruption of capillary wall, mesangiolysis, leucocytic infiltration, nuclear fragmentation, fibrinous deposits and cellular crescents are seen in about 10% of IgAN patients and in 50% of HSPN[20] (D'Amico, 2000). The loss of renal function is co-related with the extent of glomerular sclerosis and tubular atrophy with interstitial fibrosis so it parallels progressively declined functional nephrons[21]. One third of patients of IgAN experience hyaline arteriosclerosis due to associated hypertension. The grading of IgA nephropathy grading is done on chronicity based indices comprising of tubular atrophy, glomerulosclerosis, interstitial fibrosis and hyaline arteriosclerosis[22] (Lai, 2002). The organization of description of histopathological lesions in this study is based on Haas single grade classification of IgA nephropathy.

In the present study (Table: 1,2) the light microscopic examination revealed 29.4% cases each of focal mesangial proliferative GN and advanced chronic glomerulonephritis, that is in accordance with Bergers' original series of patients[18] (Berger, 1969). In the present study 2(11.8) cases i.e., one of FSGS and of membranoproliferative GN histology and 1(5.9%) case showed morphology of diffuse mesangial proliferative GN and another showed diffuse proliferative GN.

In our study, we analysed the expression of iNOS in the renal biopsy tissue by immunohistochemistry on paraffin embedded sections by using anti iNOS antibody. IHC was performed on renal biopsy tissue from the patients with IgA proliferative GN, IgA non proliferative GN, normal lung tissue as a positive control and normal kidney tissue as a negative control. Nitric oxide (NO) is synthesised from L – arginine by NO Synthases and it plays an important role in cellular injury and defence. NO is very labile and its half life is only a few seconds but when it is produced by inducible NO synthases (iNOS), its action lasts for many days. In glomerulonephritis, iNOS is induced as a part of rapid initial response to injury[23]. Many studies support that cytokines mediate induction of iNOS to produce local NO that might be involved in the initiation and / or progression of proliferative GN. iNOS regulates the T-cell dependant IgA class switching recombination through TGF-β on cells and it causes glomerular injury and expansion of mesangial cells and matrix[24]. As studied by Qiu suggests that increase interstitial expression of iNOS was associated with poor prognosis[25]. The present study reflects the same results (Table 3,4) of iNOS giving positive expression in proliferative classes of IgA i.e., focal .mesangial proliferative GN , diffuse proliferative GN, membranoproliferative GN, Diffuse mesangioproliferative GN in glomerular and tubulointerstitial region and in the end stage renal disease in tubular and interstitial regions. On the other hand non proliferative classes i.e., FSGS and membranous glomerulopathy do not show iNOS expression and these results are in accordance with the other reports[9]. In the present study there is strong association between proliferative pattern of IgA nephropathy and iNOS expression (p-value 0.000).

Our study showed that iNOS expression was associated with serum creatinine levels (p-value 0.040) (table no. 5). Hence iNOS can be detected in earlier stages of proliferative conditions as well as in late stages. Therefore it could be helpful in distinguishing the nature of GN which

would subsequently help in therapeutic options and also in predicting the prognosis.

The present study focused upon the morphological patterns of IgA nephropathy and their relationship with clinical findings with the help of histochemistry, aid of special stains, immunofluorescence and immunohisto-chemistry with iNOS. There were no such studies reported previously from Pakistan that could show the exact percentage of different morphological patterns and presentations of IgA nephropathy in Pakistan, therefore this study will be helpful in not only diagnosing the different classes of IgA nephropathy but also in differentiating the proliferative type from non proliferative type with the help of iNOS expression. This study will also be of great help in managing the classes of IgA nephropathy accordingly. It emphasized that for diagnosing IgA nephropathy, IF is an essential parameter otherwise many biopsies are likely to remain undiagnosed whereas immunohistochemistry has a direct bearing on making the right diagnosis about the nature of IgA nephropathy.

Conflict of interest: Not there.

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