Frequency and Morphology of IgA Nephropathy in Multiple Centers Lahore

ATIKA MASOOD1, MUNAZZA CHOUHARY2, FATIMA RASHID3, A.H.NAGI4.

ABSTRACT

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 Schonlein purpura 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. 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±3.14mg/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. The age range for IgA nephropathy patients was 4 to 73 years with a mean age of 31.41 years and female to male ratio was 1:1.83. IgA nephropathy patients presented with microscopic haematuria (61.5%), macroscopic haematuria ± proteinuria of less than 2g/dl and advanced chronic nephropathy and autoimmune IgA nephropathy.

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.

Key words: IgA nephropathy, Glomerulonephritis , immunofluorescence

INTRODUCTION

Glomerulonephritis (GN) comprise a varied 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 a significant cause of end stage renal disease. IgA nephropathy is described immunologically by the deposition of IgA immune complexes in the mesangium in the setting of varied clinical features, but mainly proteinuria and asymptomatic haematuria3. Although the pathogenesis of IgA nephropathy is not completely known according to the proposed pathogenesis by many researchers, they divide the IgA nephropathy in two sub types i.e., classical IgA nephropathy and autoimmune IgA nephropathy. Classical subtype evolves after glomerular injury by exogenous antigen – endogenous IgA immune complex and exogenous antigen that could be dietary4, viral5 or bacterial6. The autoimmune type is induced by aberrant, under galactosylation of IgA1 derived from subpopulation of plasma cells and B cells. The aberrant galactosylated IgA1 is recognised by the antiglycan IgG and IgA1 antibodies and resultant complexes deposit in the mesangial region causing mesangial cellular proliferation and matrix expansion8. The activated mesangial cells also produce pro--inflammatory cytokines like TGF – β, PDGF– β, chemokines and activate the alternative complement pathway7.

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. IgA nephropathy prevalence shows distinct geographic variation as it is highest in Singapore, Hong Kong, Japan, Australia, Finlad and southern Europe where it accounts for 20% -40% of cases of primary GN. While in US, England and Canada the prevalence is very low9. In a report from Pakistan it was observed that the frequency of IgA nephropathy as 12.65% in a total of 105 renal biopsy samples in which only 1 biopsy turned out to be of Henoch Schonlein nephritis (HSN). The male to female ratio is 1.5 to 110. In the previous two studies conducted in

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southern part of Pakistan, the prevalence of IgA nephropathy is 2% with application of immuno-peroxidase technique and 5.9% in another study. The study conducted in northern part of Pakistan reported 7.9% prevalence of IgAN. Recently a study was carried out by Noor in northern areas of Pakistan and according to that prevalence was reported to be 20.83%. The variation in prevalence of IgA nephropathy reflects ancestral differences, clinical policies for conducting renal biopsies and role of an incremental antigen. The annual screening of urines in Japan and Singapore in schools and army recruitment offices may contribute to the higher detection of IgAN. A community urine analysis survey done in Karachi using dipstick method showed that in asymptomatic individuals haematuria was present in 25% and proteinuria in 15%. The incidence reported in Pakistan is rightly called “Tip of the iceberg”, and actual prevalence would be higher if studies are conducted on larger population.

Light microscopy shows histological variability in this disease, ranging from minimal change to diffuse proliferative glomerulonephritis (GN) to crescentic GN. The most often Haas classification is used to grade the IgA nephropathy with Class I as Minimal Histological Lesion, Class II as FSGS, Class III as Focal proliferative GN, Class IV as diffuse proliferative GN and Class V as Advanced Chronic GN (or end stage renal disease. ESRD).

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.

**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. mean age of the male and female patients was 31.17 and 21.91 years respectively.

As regards clinical history, among the 34 patients microscopic 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 0.60mg/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 1.90 mg/dl with a mean±S.D serum bilirubin being 0.65±0.42 mg/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 ++++ (>2000mg) were seen in 2 (5.9%) patients, +++ (151-2000mg) in 13(38.2%) cases, ++ (101-150mg) in 14 (41.2%) and + (30-100mg) in 4 (11.8%) patients. 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.
Table 1: Number of detected IgAN cases confirmed by IF among 34 clinically suspected patients

<table>
<thead>
<tr>
<th>No. of IgAN Cases</th>
<th>No. of IgAN Cases</th>
<th>No. of IgAN Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal Change Disease</td>
<td>0</td>
<td>01</td>
</tr>
<tr>
<td>Focal Mesangial Proliferative Nephritis</td>
<td>5</td>
<td>09</td>
</tr>
<tr>
<td>Diffuse Mesangial Proliferative Nephritis</td>
<td>1</td>
<td>03</td>
</tr>
<tr>
<td>Focal Segmental Glomerulosclerosis</td>
<td>2</td>
<td>07</td>
</tr>
<tr>
<td>Membranoproliferative Glomerulonephritis</td>
<td>2</td>
<td>04</td>
</tr>
<tr>
<td>Membranous Glomerulopathy</td>
<td>1</td>
<td>02</td>
</tr>
<tr>
<td>Diffuse Proliferative Glomerulonephritis</td>
<td>1</td>
<td>01</td>
</tr>
<tr>
<td>End Stage Renal Disease</td>
<td>5</td>
<td>06</td>
</tr>
</tbody>
</table>

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's trichrome to see the extent of fibrosis and Jones Methenamine silver stain for the detection of changes in glomerular basement membrane (GBM). When observed microscopically, morphologically variable lesions were seen with maximum 9(26.5%) cases of focal mesangial proliferative nephritis followed by 7(20.6%) cases of FSGS, 6(17.6%) cases of ESRD, 4(11.8%) cases of MPGN, 3(8.8%) cases of diffuse mesangial proliferative nephritis and 2(5.9%) cases each of MCD and MG while 1(2.9%) cases of DPGN.

Among the 34 clinically suspected cases of IgA nephropathy, 17(50%) cases turned out to be of IgAN after morphological and IF studies. The age range for IgA nephropathy patients was 4 to 73 years with a mean age of 31.41 years and female to male ratio was 1:1.83. IgA nephropathy patients presented with microscopic haematuria (61.5%), proteinuria of less than 2g/dl (35.4%), proteinuria of more than 2g/dl (58.3%) and hypertension (41.2%) mainly. Serum IgA level was raised in 76.5% patients of IgA nephropathy. According to Hass Classification in this study, highest percentage (29.4%) turned out to be of focal mesangial proliferative GN and advanced chronic GN.

In the study we have tested the relationship between serum IgA Levels and Serum Creatinine in IgA Nephropathy Patients and it shows significant relationship. (P-Value: 0.006)

In the present study we have concluded that there is strong association between morphological patterns of glomerulonephritis (suspected cases of IgA nephropathy) and immunofluorescence with IgA. (p-value 0.05). We have also concluded that there is strong association between serum IgA levels and IgA detected by Immunofluorescence. (p-value 0.05) but there no association between serum IgA levels and intensity of IgA by immunofluorescence.

DISCUSSION

Kidneys work as the master chemists of human body. They help in keeping the body in equilibrium, controlling the condition of the blood by acting like complicated sieves which separates the life saving constituents of the body, which slip through its holes. So in doing this complex job, kidneys are disposed to a wide range of diseases, counting from infections to progressive renal failure. The most common pathological problems encountered in biopsy samples from native kidney specimen are of glomerular diseases. Glomerular diseases present in a lot of variable manners ranging from asymptomatic proteinuria, asymptomatic haematuria, acute nephritis, acute renal failure, nephrotic or nephritic syndrome, rapidly progressive nephritis which ultimately progresses to chronic renal failure. Glomerular diseases require detailed pathological evaluation by light microscopy, immunohistochemistry, immunofluorescence study of biopsy as well as clinical evaluation and serological to establish a diagnosis.

IgA nephropathy (IgAN) is considered to be the most common form worldwide especially in Europe.
and some Asian countries. 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 immunohistochemistry (IH) and renal biopsy examination.

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. 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–Schönlein Purpura associated Nephritis (HSPN) and our results are in accordance with the previous studies reported from Pakistan 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. The study from the northern parts of Pakistan showed the prevalence of IgA nephropathy to be 7.9%. In India the incidence of 8.9% and 14.26% were reported.

The age range in the present study was 4–73 years with a mean age of 31.41 years. The female to male ratio in this study was 1:1.83, which is in accordance with the previous reports. The salient features of our clinical findings include history of microscopic / macroscopic haematuria, proteinuria, hypertension, body / facial oedema etc. The features which present worst prognosis include males, older age, obesity, insulin resistance, microscopic haematuria, serum creatinine level, decline in GFR, proteinuria >1000 mg/day, Albuminuria>30mg/day, hypertriglyceridaemia, hypercholesterolaemia and increased uric acid level. A large number of patients (76%) in this study gave a history of haematuria, out of which 61.5% showed microscopical while 38.4% showed macroscopical haematuria. These findings are in complete harmony with most of earlier studies in this region.

In the present study, 35.4% patients had proteinuria of less than 2g/day while 58.3% had proteinuria of more than 2g/day which is in accordance with reports from United States, India and Pakistan while studies conducted in Japan showed contradiction perhaps due to country’s annual urine screening programme.

Among all, 41.2% patients presented with hypertension in our study which is in accordance with the other studies and cohort study from Saint-Etienne region.

In the present study we observed that the mean level of serum creatinine was 3.512±3.58 and in 58.8% that is more than 1.2mg/dl which is according to the previous studies. The serum IgA level was raised in 76.5% patients in the present study. The previous studies showed a significant elevation of serum IgA in more than 50% cases but the elevation in serum IgA level is not a consistent finding to be used as a diagnostic marker for IgA nephropathy. In the present study we observed that there was strong association between serum IgA levels and IgA detected by Immunofluorescence (p-value 0.037) however there was no strong association between serum IgA levels and intensity of IgA by immunofluorescence (p-value 0.062). Serum IgA levels have no significant prognostic value for progression or severity of disease. As in the previous studies the present work also showed that it cannot be used as an alternative to renal biopsy for the diagnosis of IgA nephropathy. Many candidates for a serological diagnostic marker of IgA nephropathy, such as increased levels of serum IgA or of IgA containing complexes with fibronectin, IgG, or C3, have been previously examined and the serum IgA concentration is significantly elevated in about half of the patients with IgA nephropathy patients but this parameter is not satisfactory as a diagnostic test alone for diagnosing IgA nephropathy and is the association with mesangial deposits in glomeruli, as also confirmed by our study.

Light microscopy shows histological variability in IgA nephropathy, ranging from minimal change to diffuse proliferative glomerulonephritis (GN) to crescentic GN. Mesangial hypercellularity is mainly focal segmental and sometimes it is accompanied by mesangial matrix expansion. Necrotizing lesions like disruption of capillary wall, mesangiolysis, leucocytic infiltration, nuclear fragmentation, fibrous deposits and cellular crescents are seen in about 10% of IgAN patients and in 50% of HSPN. 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. 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. Due to diverse histological nature of IgA nephropathy, several classification systems, i.e., semiquantitative and single grade are in use. According to the recent survey of members of renal pathology society, it was seen that most histopathologists use single grade system, which combines glomerular and tubulointerstitial features. The organization of description of histopathological lesion in this study is based on Haas single grade classification of IgA nephropathy.

In the present study (Table 2.3) 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 (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 IgA nephropathy there is a morphological variation from minimal change to diffuse proliferative and advanced chronic GN.

According to Haas classification, the class II FSGS similar to our study represent 2(11.8%) cases which are in accordance with the previous studies. Among two cases, one showed proteinuria of 2g/day and the other had no history of proteinuria, whereas serum creatinine levels were more than 1.2mg/dl in both cases. The microscopic features are in complete harmony to Haas and showed hyalinisation in mesangial area less than 50% and adhesions of glomeruli with capsule. Fibrocellular crescents and sclerosis in glomeruli were also present along with capillary wall thickening. There were necrotic changes in tubules and casts with moderate type of inflammation and fibrosis in interstitium. The immunofluorescence microscopy showed IgA with IgG in one case while in another case IgM and C3 were detected in both cases.

The focal mesangial proliferative GN was 29.4% in our study and histological findings showed focal segmental increase in mesangial cellularity and the matrix showed adhesions to capsule. Along with this tubular atrophic changes and mild to moderate interstitial inflammation were seen and these changes are consistent with class III of Haas and grade II of Lee classifications. Among 5 cases of IgA nephropathy, IgG was present in two cases, IgM in only one case and C3 in three cases along with IgA. In two cases IgA was the sole detector of IgA nephropathy.

Class IV of Haas encompasses changes of diffuse mesangioproliferative GN, membrano-proliferative like changes and diffuse proliferative GN. The histological changes seen in our study were glomerular hypercellularity of mesangial/endothelial, crescent formation, necrotic changes, in more than 50% of glomeruli. Tubulointerstitial changes were seen in mild to moderate type and in accordance with Haas class IV changes. IgA was sole detector in one case while in IgG in three cases and with C3 in two cases. Among the membranoproliferative, one case turned out to be Henoch-Schonlein purpura nephritis, which showed changes according to class VI of ISKDC.

In the present study 29.4% cases turned out to be advanced chronic GN and histological changes seen in our study were global hyalinisation in mesangial, crescent, adhesions with capsules, sclerosis of glomeruli, tubular atrophic changes and moderate to severe type interstitial fibrosis and inflammation. These findings are consistent with Haas class V. The tubular atrophy is a sign of regression of renal function and intraepithelial lymphocytes in renal tubules are used as an initial marker for progression of IgAN towards renal failure. This leads to an end stage renal failure in 20%-40% of patients. IgA was solely detected in one case, in two cases with IgG and in four cases with C3.

In the present study, one case of membranous GN co existing with IgA nephropathy was detected and it had also been previously reported by other workers. The biopsy showed histological changes of membranous nephropathy with variable thickness of glomerular capillary walls and on Jones methanamine silver (JMS) that basement membranes showed spikes along the epithelial aspect. The immunofluorescence detected IgG and IgM along with IgA and C3 component.

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 as described above. 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 (Bergers, 1969).
In the present study there is strong association between morphological patterns of glomerulonephritis (suspected cases of IgA nephropathy) and immunofluorescence with IgA was observed (p-value 0.046). IF frozen section remains a powerful tool in distinguishing various nephropathies and it has proven better than IHC using immunoglobulin (Ig) on paraffin embedded sections. Studies conducted by Wagrowska-Danilewicz showed that direct IF on paraffin embedded sections (IF-P) is a less sensitive method than IF on frozen sections, hence the number of cases of glomerulopathies may be overlooked. The rate of agreement between IF-P and IF-F with respect to the presence of IgM was 44.4%, IgA – 56.5%, C3-51.5% and IgG – 73.9%. Therefore IF-P was less sensitive than IF-F for the detection of C3 and Ig in all glomerulopathies.

In the present study, the IgA deposits were the characteristic and defining feature of the disease and they were accompanied by IgG in 9(52.9%), IgM in 3(17.64%) and C3 in 12(70.58%) patients in a dominant manner in 11(64.70%) cases and in co dominant in 6(35.29%) cases. While IgA deposits were exclusive in 4(23.5%) patients and these results are in accordance with the previous studies. IgA may be the sole immunoglobulin (Ig) or may be dominant or co dominant Ig with co deposits of IgG or IgM or both. The IgA deposits were primarily detected in granular pattern in mesangial region in 14(82.35%) cases and along capillary membrane in 3 (17.6%) patients. It is also in accordance with the previous studies.

Searching the literature on IgA nephropathy from Pakistan reveals that very little work is available on this topic. There are however few studies on the prevalence of IgA nephropathy in Pakistan. Earlier renal biopsy studies were based on light microscopy only and they missed IgA diagnosis in many patients. Recently, a few centers have performed IHC examination on renal biopsies hence cases of IgA nephropathy were reported. The first study to report on IgA nephropathy in Pakistan was published in 1988 by Khan et al in which 50 cases of renal biopsies were stained using immunoperoxidase method and frequency was reported to be 21%. Later, the same authors reported a series of 102 renal biopsies, in which IgA nephropathy was observed in 5.9%. Lakhnana et al in 1995 made a report of northern part of the country, in which IgA nephropathy was 7.9% of the cases. Recently, a prevalence of 12.65% by Muzaffar et al and 20.83% by Noor et al has been reported from different centres. It is important to note that the data of clinical presentation of IgA nephropathy reported in the previous studies from Pakistan was rather flimsy. The present study, to our knowledge is the first study, encompassing clinical, biochemical, morphological and importantly immunofluorescence and immunohistochemical details of IgA nephropathy from Pakistan.

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 and immunofluorescence. 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 diagnosing the different classes of IgA nephropathy. It emphasised that for diagnosing IgA nephropathy, IF is an essential parameter otherwise many biopsies are likely to remain undiagnosed.

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