

Clinical, Histopathological and Immunofluorescent Findings of IgA Nephropathy

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ABSTRACT

Background: IgA nephropathy, a prevalent disease in Asia, is considered the main cause of end stage renal disease among primary glomerular disease. **Objective:** To determine the frequency of different clinical, histopathological and immunofluorescent characteristics of IgA nephropathy. **Methods:** Renal biopsies of 376 patients were received for immunofluorescent and for histopathological studies. Biopsies were stained with fluoresce isothiocyanate (FITC) labeled antibodies against IgG, IgA, IgM, C3, C4 and fibrinogen for fluorescent microscopy. For histopathological examination, the specimens were stained with hematoxylin and eosin, periodic acid schiff and methanamine silver stains for light microscopy. **Results:** IgA nephropathy was diagnosed in 39 cases (10.4%) with a mean age 31.5 years and a male to female ratio of 2.8:1. The disease was observed in 11(29.7%) patients aged 21-30 years, followed by 8 patients (21.6%) aged 11-20 years group. Nephrotic range proteinuria was the most common laboratory finding which was detected in 11 patients (37%). Mesangioproliferative glomerulonephritis was the most common histopathological finding which was found in 7 patients (35%). IgA with other immunoglobulins and complements were deposited in 28 specimens (71.8%) as detected by immunofluorescence. **Conclusion:** IgA nephropathy is common in young people and one third of it results in end stage renal disease. We suggest that Immunofluorescent assay can be considered for the conclusive diagnosis of IgA nephropathy in young patients presenting with proteinuria/hematuria.

Keywords: Glomerulonephritis, IgA Nephropathy, Immunofluorescence

INTRODUCTION

IgA nephropathy was first described in 1968 (1) and is the most common type of glomerulonephritis worldwide (2). Its prevalence varies across the globe, ranging from

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less than 10% in United States to 30-40% in Asia (3). It is a disease of young males and commonly presents itself with episodic macroscopic hematuria, persistent or intermittent microscopic hematuria or with chronic renal failure and hypertension. Light microscopy usually reveals mesangioproliferative glomerulonephritis though other findings like focal proliferative glomerulonephritis, necrotizing lesions or crescents are not uncommon. The major finding is the presence of IgA deposits along with complement components and other immunoglobulins in the glomeruli detected by immunofluorescence (4). Abnormal glycosylation of IgA has been found to be the defect leading to its prolonged survival in circulation and deposition in the glomeruli, thus activating the complement and causing glomerular injury. Although no satisfactory treatment is available for the disease, ϕ -3 fatty acids, steroids and immunosuppressive agents have been used with variable success (4). Initially considered a benign disorder, IgA nephropathy is now the main cause of end stage renal disease among primary glomerular diseases, with 60-70% of the patients progressing to chronic renal insufficiency and 20-30% developing end stage disease, eventually requiring renal replacement therapy (4,5).

MATERIALS AND METHODS

This descriptive study was carried out at the departments of immunology and histopathology, Armed Forces Institute of Pathology, Rawalpindi, from Jan 2009 to Dec 2009. Renal biopsy specimen of 376 patients either in normal saline for immunofluorescence in 10% buffered formalin for histopathology were received in this period. All the biopsies received were included in the study and there were no exclusion criteria except when the biopsy for immunofluorescence was sent in formalin, and thus they were excluded from the study. The study protocol was approved by ethical and research committee of the Armed Forces Institute of Pathology.

The clinical features were defined as: nephrotic range proteinuria (>3.5 g/24 hr), non-nephrotic range proteinuria (<3.5 g/24 hr), proteinuria (presence of 1+ or more protein in routine urine examination), hematuria (presence of RBCs in routine urine examination), hypertension (BP $>140/90$ mm of Hg) and chronic renal failure (persistently raised blood urea and creatinine with hypertension/proteinuria) (6). For direct immunofluorescence, biopsies were snap frozen and 4-5 μ m thick sections were cut on cryostat and air dried. These were then stained with fluorescein isothiocyanate (FITC) labeled antibodies against IgG, IgA, IgM, C3, C4 and fibrinogen (Diasorin, USA). Stained specimens were then observed under fluorescent microscope. Fluorescence intensity was graded as mild, moderate or bright.

For histopathological examination, biopsies were fixed in 10% formalin and processed for paraffin embedding, followed by preparing 3-5 μ m thick sections and staining with hematoxylin and eosin, periodic acid schiff and methanamine silver stains for microscopy. The patterns were classified morphologically (7) by histopathologists unaware of the immunofluorescent findings.

All data were analyzed by SPSS 13.0 for age group frequencies (every 10 years), gender, clinical/laboratory features, histopathological findings and immunofluorescence results.

RESULTS

Out of 376 renal biopsies received for immunofluorescent studies, 39 patients were identified as IgA nephropathy (10.4%). These included 28 males and 10 females (2.8:1, gender of one patient was not available). Their mean age was 31.5 years (range 10-65 years). They were then divided into various age groups and the frequency of IgA nephropathy in each age group was calculated (Figure 1).

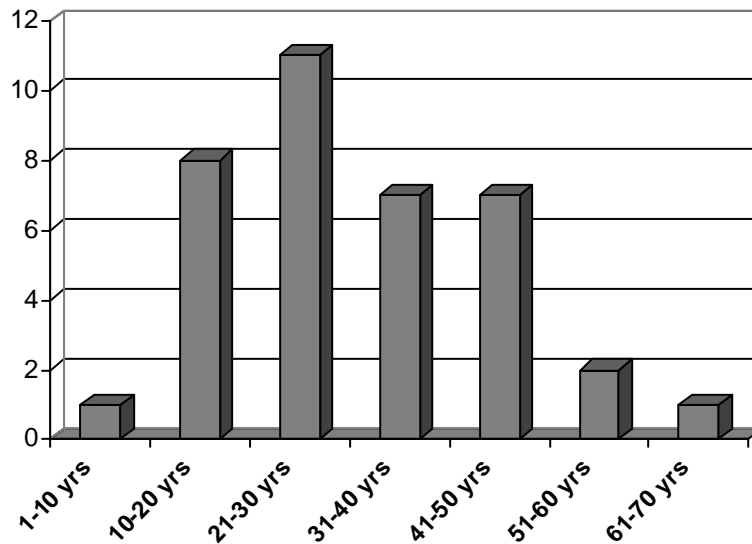


Figure 1. Frequency of IgA nephropathy according to age groups (n=37, missing=2).

Majority of the patients presented with nephrotic range proteinuria (37%) followed by hematuria with proteinuria (27%). The most common histopathological finding was mesangioproliferative glomerulonephritis (35%), though a large number of cases remained undiagnosed on histopathology (25%) and required immunofluorescent studies for diagnosis.

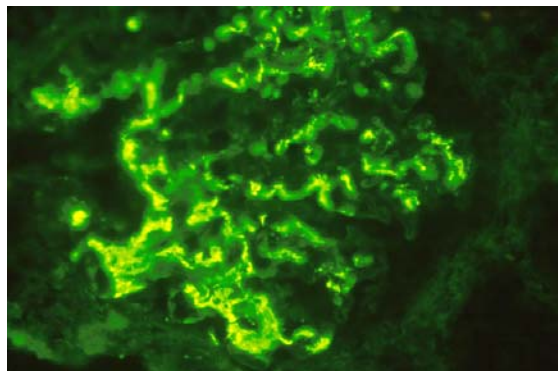


Figure 2. IgA deposits in mesangium (Immunofluorescence).

Deposition of IgA with IgM/IgG and C3 complement in the mesangial region of glomeruli was the most frequent finding (71.8%) of immunofluorescence (Figure 2). Laboratory features, histopathological and immunofluorescent findings are summarized in Table 1.

Table 1. Frequency of laboratory features, histopathological and immunofluorescence findings.

Characteristic (n=39)	Finding	Number (Valid percentage)
Main clinical/laboratory feature (n=30, missing=9)	Nephrotic range proteinuria	11 (37%)
	Non-nephrotic range proteinuria	4 (13%)
	Hematuria + proteinuria	8 (27%)
	Macroscopic hematuria	2 (7%)
	Microscopic hematuria	2 (7%)
	Hypertension	1 (3%)
	Chronic renal failure	2 (6%)
Histopathological findings (n=20, missing=19)	Mesangioproliferative GN	7 (35%)
	Non diagnostic	5 (25%)
	Membranoproliferative GN	4 (20%)
	Rapidly progressive GN	2 (10%)
	Crescents formation	1 (5%)
	Rejection	1 (5%)
Immunofluorescence deposits (n=39)	IgA + IgM/IgG + C3	28 (71.8%)
	IgA + IgM/IgG	5 (12.8%)
	IgA + C3	4 (10.3%)
	IgA	2 (5.1%)

DISCUSSION

IgA nephropathy is the commonest form of primary glomerulonephritis and the major cause of end stage kidney disease worldwide. Its prevalence varies in different parts of the world ranging from 2-52% of all renal biopsies (8). In our region, it is reported to be 14.26% in India (6), 11.9% in Bangladesh (9) and 10.2% in Saudi Arabia (10). In Pakistan, its prevalence was initially reported to be 5.9% (11), but a relatively recent study showed it to be higher, nearing that of other countries in the region, i.e. 12.65% (12). Later it was shown to be even higher in North West Frontier province (13). Among 376 renal biopsies studied by us, its prevalence matched the above figures

(10.4%). However, these figures vary considerably and the difference between the regions can be due to differing referral practices and biopsy criteria (14). Additionally, its diagnosis requires immunofluorescence facilities that are relatively scarce in developing countries.

The disease was more common in males (2.8:1) which is consistent with international findings (2:1 in Japan to 6:1 in Europe and United States) (5), although smaller ratios (approx 1.2:1) have been reported in regional studies (8,10). In addition, we calculated its frequency in various age groups and found that young people are at increased risk of the disease; it was highest in 21-30 year old subjects (29.7%) followed by the 11-20 year old ones (21.6%), while at both extremes of age, only one case (2.7%) was noted. Similar results were obtained in international studies, reporting its highest incidence in the second and the third decades of life (5).

Exact pathogenesis of the disease has not been elucidated so far. Increased production of IgA1 and its defective galactosylation has been considered the inciting event. Other mechanisms include decreased hepatic clearance of IgA1 and decreased activity of β 1, 3-galactosyl transferase that normally adds galactose to IgA1 molecule. Defectively galactosylated IgA deposits in mesangial cells followed by the release of cytokines and growth factors and the culmination of renal injury (4,5,15).

Majority of our patients were presented with nephrotic range proteinuria (38%) followed by hematuria + proteinuria (27%). Although most of the subjects remain asymptomatic and diagnosis usually follows routine analysis, episodic macroscopic hematuria is considered to be the main presenting problem (4). Muzaffar et al. have shown nephrotic range proteinuria to be the most common presentation (40%) (12), coinciding with our findings and suggesting that IgA nephropathy may differ in its presentation in different regions. Similarly, nephrotic range proteinuria has been shown to be the most common presentation in India as well (16). The underlying pathophysiology of the disease encompasses a whole array of immunological alterations, including increased production of aberrantly glycosylated IgA by an abnormal clone of B lymphocytes, decreased clearance, host's immune response to the abnormal immunoglobulin and the abnormal accumulation of IgA1 in mesangium (17). Thus the subjects in different geographical regions may respond to the disease in different manners, resulting in different clinical manifestations as depicted in clinical findings of our and other regional studies, and it should be given due consideration while deciding renal biopsy.

Mesangioproliferative glomerulonephritis is the main histopathological finding worldwide. 35% of our biopsies showed mesangial proliferation. However no single histopathological finding is the characteristic of IgA nephropathy and mesangial proliferation may also be observed in a variety of other glomerular lesions. Similarly IgA nephropathy may also show diffuse endocapillary proliferation, segmental sclerosis, membranoproliferative glomerulonephritis or crescents formation (5). Confirmation can only be achieved by the immunofluorescence of the biopsies. Majority of biopsies are expected to contain immunoglobulins along with IgA, though IgA deposition must predominate the others for the diagnosis to be certain (18,19). 72% of our biopsies showed the deposition of IgA along with IgM/IgG and C3 while isolated deposition of IgA was observed in only 2 (5%) biopsies. IgG/IgM was co-deposited with IgA in 33 (84.6%) of our biopsies and C3 co-deposition with IgA was noted in 32 subjects (82.1%). Muzaffar et al. have reported that IgG/IgM is deposited in only 30% of the cases (12), though they studied only 10 cases of the disease. Besides, Moriyama

et al. have documented 80% of the cases (307 of 384) (20) and van Es has reported the majority (83% IgG and 65% IgM) of biopsies to contain IgG/IgM along with IgA (21). These IgA deposits are chiefly accumulated in mesangial regions of glomeruli while focal paramesangial or subendothelial extensions may also be present. As far as the complement deposition is concerned, the capacity of human IgA to activate complement remains a controversial subject. Several studies have shown that aberrantly glycosylated IgA1 in IgA nephropathy is capable of activating the alternate pathway (and not the classical pathway) resulting in C3 (and properdin) deposition in mesangium along with immunoglobulins (21,22,23).

However, there is no single non-invasive test that carries enough sensitivity and specificity to avoid a renal biopsy for IgA nephropathy diagnosis (24,25). Secondly there is no single characteristic histopathological feature for the disease and diagnosis requires fluorescence facilities (5) that are not easily available especially in developing countries. Thirdly, 25-40% of the patients invariably succumb to end stage renal disease requiring renal replacement therapy (4,5). All these factors together make IgA nephropathy an entity that physicians should keep in mind while dealing with patients having hematuria/proteinuria.

In conclusion, IgA nephropathy, as an immune complex mediated disorder, is the commonest form of primary glomerulonephritis that is defined immunohistologically by the presence of glomerular IgA deposits. Since it is more common in young adults and requires special diagnostic facilities, patients with hematuria and proteinuria should be carefully assessed for the requirement of renal biopsy and immunofluorescence.

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REFERENCES

- Berger J, Hinglais N. Intercapillary deposits of IgA-IgG. *J Urol Nephrol.* 1968; 74:694-5.
- Julian BA, Waldo FB, Rifai A, Mesteckey J. IgA nephropathy, the most common glomerulonephritis worldwide: a neglected disease in the United States? *Am J Med.* 1988; 84:129-32.
- Chapel H, Haeney M, Misbah S, Snowden N. *Essentials of clinical immunology.* 4th ed. Blackwell science ltd; 2001.
- Olson JL. Renal disease. In: Parslow TG, Stites DP, Terr AI, Imboden JB. *Medical Immunology.* 10th ed. United States: Lange Medical Books/McGraw-Hill Medical Publishing Division. 2003; 481-94.
- Donadio JV, Grande JP. IgA nephropathy. *N Engl J Med.* 2002; 347:738-48.
- Goddard J, Turner AN, Cumming AD, Stewart LH. *Kidney and Urinary Tract Diseases.* In: Boon NA, Colledge NR, Walker BR, Hunter JAA. *Davidson's Principles and Practice of Medicine.* 20th ed. Churchill Livingstone/ Elsevier Limited. 2006; 455-518.
- Rosai J, Ordonez NG. Urinary tract. In: Ackerman LV, Rosai J. *Rosai and Ackerman's surgical pathology.* 9th ed. St Louis: MOSBY. 2005;1163-359
- Chandrika BK. IgA nephropathy in Kerala, India: A retrospective study. *Indian J Pathol Microbiol.* 2009; 52:14-6
- Sharmin F, Khan BR, Rahman M, Rashid HU, Islam B, Kamal M, et al. IgA nephropathy in teaching hospitals of Dhaka. *Bangladesh Medical Research Council Bulletin.* 1997; 23:25-9.
- Mitwalli AH, Al Wakeel J, Abu-Aisha H, Alam A, Al-Sohaibaini M, Tarif N, et al. Prevalence of glomerular diseases: King Khalid university hospital, Saudi Arabia. *Saudi J Kidney Dis Transpl.* 2000; 11:442-8.
- Khan TN, Sinniah R, Naqvi SA, Lal M, Osmani M. IgA nephropathy in Pakistan. *J Pak Med Assoc.* 1990; 40:31-6.
- Muzaffar S, Azad NS, Kayani N, Pervaz S, Ahmed A, Hasan SH. The frequency of IgA nephropathy at a single centre in Pakistan. *J Pak Med Assoc.* 2003; 53:301-5.

- 13 Noor M, Jamal SM, Mohammad W, Shah D. IgA nephropathy in North West Frontier province of Pakistan. *Gomal J Med Sci.* 2007; 5:62.
- 14 Maxwell PH, Wang Y. Genetic studies of IgA nephropathy. *Nephron Exp Nephrol.* 2006; 102:76-80.
- 15 Suzuki H, Moldoveanu Z, Hall S, Brown R, Vu HL, Novak L, et al. IgA1 secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. *J Clin Invest.* 2008; 118:629-39.
- 16 Chacko b, John GT, Neelakantan N, Korula A, Balakrishnan N, Kieubakaran NG, et al. Presentation, prognosis and outcome of IgA nephropathy in Indian adults. *Nephrology.* 2005; 10:496-503.
- 17 Mubarak M. IgA nephropathy: An update on pathogenesis and classification. *JCPSP.* 2011; 12:230-3.
- 18 Hricik DE, Chung-Park M, Sedor JR. Glomerulonephritis. *N Engl J Med.* 1998; 339:888-99.
- 19 Orth SR, Ritz E. The nephrotic syndrome. *N Engl J Med.* 1998; 338:1202-11.
- 20 Moriyama T, Shimizu A, Takei T, Uchida K, Honda K, Nitta K. Characteristics of immunoglobulin A nephropathy with mesangial immunoglobulin G and immunoglobulin M deposition. *Nephrology.* 2010; 15:747-54.
- 21 Van Es LA. Pathogenesis of IgA nephropathy. *Kidney International.* 1992; 41:1720-9.
- 22 Wayatt RJ, Kanayama Y, Julian BA, Negoro N, Sugimoto S, Hudson EC, et al. Complement activation in IgA nephropathy. *Kidney International.* 1987; 31:1019-23.
- 23 Wayatt RJ, Julian BA. Activation of complement in IgA nephropathy. *American Journal of Kidney Diseases.* 1988; 12:437-42.
- 24 Floege J, Feehally J. IgA nephropathy: Recent developments. *J Am Soc Nephrol.* 2000; 11:2395-403.
- 25 Madaio MP, Harrington JT. The diagnosis of glomerular diseases. *Arch Intern Med.* 2001; 161:25-34.