Role of immunoflourescence in the diagnosis of glomerulonephritis

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Abstract

Objective: To correlate the findings of immunoflorescence (IF) with morphology in renal biopsies of patients with glomerulonephritis (GN) of both primary and secondary nature.

Methods: The cross-sectional analytical study was conducted at the Shifa International Hospital's Department of Pathology form March 2007 to August 2008, during which a total of 207 renal biopsies were done. Of them, the study included 92 cases which were diagnosed as primary or secondary glomerulonephritis under light microscope. Those cases were selected in which both light microscopy (LM) and immunoflorescence were done.

Results: Of the 92 patients, 79 (85.8%) were adults (≥ 19 years) and 13 (14%) were children (< 19 years). The mean age of adults was 36.44 ± 11.55 (range19-69 years) and that of the children was 10.54 ± 3.85 years (range 4-18years). immunoflorescence changed the morphologic diagnosis in 20(21.73%) cases. The pattern of disease was: membranous glomerulonephritis in 24%, focal segmental glomerulosclerosis (FSGS) in 18.4%, mesangiocapillary glomerulonephritis in 2%, and minimal change disease (MCD)in 16% of the cases.

Conclusion: Light microscopy alone can misdiagnose renal disease. This is especially important in cases of early stage membranous, IgA nephropathy (IgAN), Lupus nephritis and IgM nephropathy (IgMN), as these entities can only be diagnosed by correlating the microscopic, immunoflorescence findings and clinical details.

Keywords: Renal biopsy, Light microscopy, IF, Electron microscopy, MCD, Membranous GN, FSGS, IgAN, Lupus nephritis (JPMA 62: 240; 2012).

Introduction

Kidney reacts to various injurious agents in a limited number of ways or patterns. The determination of type and pattern of renal insult is necessary to determine the clinical course of the disease. In renal pathology, essentially a combination of light microscopy (LM), immunoflorescence (IF) and electron microscopy (EM) are necessary for arriving at a particular diagnosis.[1] It is needless to emphasise the importance of clinical and laboratory data and no renal biopsy should be read without the relevant clinical data. Laboratory data includes 24 hours urine protein estimation, renal function tests, antinuclear antibody (ANA), anti double standard DNA (anti-dsDNA), complement components (C3, C4), hepatitis B surface antigen (HSsAg), and hepatitis C antibody (Anti HCV).

The list of primary and secondary glomerulonephritis (GN) is long and commonly includes diseases like minimal change disease (MCD), membranous GN, FSGS, diffuse endocapillary (post infectious) GN, IgAN, diabetic nephropathy and lupus nephritis.[2] These diseases present as one of the following clinical syndromes: nephritic syndrome, nephrotic syndrome(NS), non-nephrotic proteinuria, isolated haematuria and acute or chronic renal failure.[3,4] The purpose of this study was to highlight the importance of IF in accurately diagnosing various types of GN.

In developing countries, the diagnosis of renal biopsy is often based on LM. If IF is not incorporated, then this practice often leads to misdiagnosis.[5-8] In this study, renal biopsies were evaluated after taking into account detailed clinical history, serologic findings, LM findings and IF results. A similar study done at the Sindh Institute of Urology and Transplantation (SIUT), Karachi, evaluated renal biopsies taking into account all these parameters.[1] Their data presented disease pattern in southern Pakistan. To our knowledge, this is the first study representing disease pattern with reference to aforementioned parameters in the population of northwest and central Pakistan. All previous studies done in this region evaluated only morphologic patterns including a study carried out at the Pakistan Institute of Medical Sciences, Islamabad.[5-9]

Patients and Methods

All the 207 renal biopsies reported in the Histopathology section at the Department of Pathology in Shifa International Hospital, Islamabad, from March 2007 to August 2008, were retrieved .The selected cases (92) were those in which LM and IF had been done. Only those cases in which a separate renal core or a portion of same renal biopsy was processed for IF were included.

The cases without GN (115) were excluded. These

included those with Tubulointerstitial Nephristis 30 (26%); End Stage Renal Disease 23 (20%); Pyelonephritis 18 (15.65%); Hypertensive Nephropathy 15 (13%); Diabetic Nephropathy 15 (13%); Acute Tubular Necrosis 8 (6.9%); No Pathological Diagnosis 8 (6.9%); Tuberculosis 2 (1.7%); and Oxalosis 1 (0.86%) nephropathy were excluded.

Adequate clinical details were also recorded in a separate pro-forma. In each case multiple thin sections (3 microns) were cut for light microscopy after fixation in 10% formalin followed by special stains like Periodic Acid Schiff, Gomori methenamine silver and Trichrome. In most cases a separate core was received in proper transport medium (Zeus Fixative, procured from Zeus scientific, Branchberg, New Jersey, 08876, USA). Antibodies to IgG, IgM, IgA, C3, C4, Fibrinogen and C1q were procured from Binding Site. The final diagnosis was rendered after correlating clinical data with LM and IF findings.

The data was analysed on Statistical Package for Social Sciences (SPSS) Version 11(IBM, United States of America). Descriptive statistics such as mean ± SD for continuous variables and percentages were used for categorical data.

Results

A total of 207 renal biopsies were retrieved from the records, of which 92 cases were included in the study, that had primary or secondary GN according to our criteria. Of the 92 patients, 79 (85.8%) were adults (≥ 19 years) and 13 (14%) children (< 19 years). Mean age of the adults was 36.44 ± 11.55 (range19-69 years) and that of the children was 10.54±3.85 (range 4-19years). There were 53 (57.6%) males and 39 (42.4%) females which were then distributed according to the final diagnosis (Table-1).

The mean 24 hours urinary protein in children was 5.73±0.56gm/24 hrs (range 4.30-6.9) whereas in adults the mean value was 6.44±1.16-gm/24 hours (range 4.80-10.2gms/24 hours). ANA was positive in 10(4.83%) cases; Anti-dsDNA in 8 (3.83%) cases; Low C3 in 11(5.31%) cases; Low C4 in 10(4.83%) cases; HBsAg in 20(9.66%) cases; and Anti HCV in 8 (3.86%) cases.

Table-1: Division of glomerulonephritis cases (n= 92).

Disease	Number of Cases 'n'	Percentage %
Membranous Glomerulonephritis	22	24%
Focal Segmental Glomerulosclerosis	17	18.4%
Minimal Change Disease	15	16%
IgA Nephropathy	9	9.7%
Diabetic Nephropathy	5	5.9%
Lupus Nephritis	5	5.4%
Diffuse Glomerulosclerosis	4	4.3%
Amyloidosis	4	4.3%
Post Infectious Glomerulonephritis	4	4.3%
Crescentic Glomerulonephritis	3	3.2%
IgM Nephropathy	2	2%
Mesangiocapillary Glomerulonephritis	2	2%

IgA: Immunoglobulin A. IgM: Immunoglobulin M.

Of the 92, IF confirmed the provisional diagnosis of LM in 59 (64.1%) cases, whereas in 20 (21.73%) cases the diagnosis was finalised after correlating the findings of IF with morphology. Out of these 20 cases, IF eventually helped in diagnosing 9 (45%) cases of IgAN, 4 (20%) of early stage membranous GN, 4 (20%) as LN, and 2 (10%) as IgMN (Table-2).

The study found that IgAN can exhibit a number of morphological patterns, including MCD (n=2), mesangioproliferative/mesangial expansion (n=4), Focal proliferative (n=1), diffuse glomerulosclerosis (n=1), and crescentic GN (n=1).

Similarly, early stage membranous GN could have been missed in four cases that showed patterns of MCD (n=2) and mesangial expansion (n=2), if only LM had been taken

Table-2: Glomerulonephritis with different immunofluorescence findings (n = 20).

Immunoflorescence diagnosis	Light Microscopy	Number
IgA	1) Mesangioproliferative /Mesangial Expansion Glomerulonephritis	4
	2) Minimal change Disease	2
	3) DiffuseGlomerulosclerosis	1
	4) Focal Proliferative Glomerulonephritis	1
	5) Crescentic Glomerulonephritis	1
Early Stage Membranous	1) Minimal Change	2
	2) Mesangial Expansion	2
Lupus Nephritis	1) Minimal change	2
	2) Mesangioproliferative glomerulonephritis	2
	3) Focal Segmental Glomerulosclerosis	1
IgM Nephropathy	4) Minimal change	1
	5) Mesangioproliferative glomerulonephritis	1

Table-3: Glomerulonephritis showing change in morphologic diagnosis after if (n = 20).

Morphological pattern	Diagnosis after correlation with IF
Minimal change Disease (n = 7)	IgA N(n = 2) Early Stage Membranous GN (n=2) Lupus Nephritis (n=2) IgM nephropathy (n= 1)
Mesangial expansion /Mesangioproliferative Glomerulonephritis /(n = 9)	IgA nephropathy (n= 4) Early Membranous Glomerulonephritis (n = 2) Lupus Nephritis (n=2) IgM nephropathy (n= 1)
Focal proliferative Glomerulonephritis (n = 1)	IgA nephropathy (n= 1)
Diffuse glomerulosclerosis (n = 1)	IgA nephropathy (n= 1)
Crescentic Glomerulonephritis (n=1)	IgA nephropathy (n= 1)
Focal Segmental Glomerulosclerosis (n= 1)	Lupus Nephritis (n=1)

into consideration.

Five cases of LN presented morphologically as MCD (n=2), Mesangioproliferative pattern (n=2) and FSGS (n=1). Two cases of IgMN showed MCD (n=1) and. Mesangioproliferative pattern (n=1) (Table-3).

IF was helpful in diagnosing 22 cases of Membranous GN (24%), 17 of FSGS (18.4%) and MCD 15(16%).

Discussion

GN constitutes a burden on the already overwrought health services in the developing countries. Early diagnosis and treatment can prevent long-term complications.

Percutaneous needle biopsy of kidney forms an indispensable element in investigation of NS.[10] Developed countries have all the facilities needed for correct diagnosis of renal biopsy.[1] These include LM, IF and EM examination. However, the situation is quite different in developing countries. In countries like Pakistan, renal biopsies are mostly diagnosed using LM alone.[10] EM is not available at most centres and requires a whole separate setup with cost involvement. However, IF, when combined with LM, gives accurate results in most cases despite the lack of availability of EM.[11]

In majority of cases of GN, LM alone can lead to misdiagnosis.[12] LM provides morphologic pattern of GN, but specific diagnosis of glomerular disease requires integration of clinical data, IF and EM.[13]

In our study it was noted that most common GN diagnosed on renal biopsies was membranous GN followed by FSGS and MCD. Studies in the past, including local studies, show, however, the highest prevalence to be that of FSGS.[14-16]

There was no change in the diagnosis of membranous GN, the most common pattern on LM. It showed glomerular basement membrane thickening [spikes on gomori methenamine silver (GMS) staining]. All of these cases showed strong granular Glomerular Basement Membrane (GBM) deposition of IgG and C3. This finding is similar to other studies done locally.[9-13]

There was no change seen in the diagnosis of FSGS on LM and IF. FSGS showed non-immune trapping of antibody IgM in 7 (40%) cases and C3 in 3 (20%) cases. This finding is similar to a local study by Abbas et al in which FSGS cases showed focal positivity of IgM in 87.3% and C3 in 83.63% of cases.[1]

Third common pattern seen on LM was MCD (n: 15) 16%. IF showed negative results for immunoglobulin and complement. This is similar to previous studies.[17,18]

Our data shows that IF was crucial in establishing diagnosis of 20 cases (21.3%). This finding is similar to a local study by Abbas K.[1] These cases included 9 of IgAN, 4 of early stage membranous, 4 of Lupus Nephritis and 2 of IgMN.

Nine cases of IgAN had different morphologic patterns, including mesangial expansion/proliferation in 4, minimal changes in 2, and one case each of focal proliferative, diffuse glomerulosclerosis and crescentic GN. These cases could have been misdiagnosed if only morphologic pattern was taken into account. This finding is similar to other local studies.[18] IgAN is the most common GN worldwide.[17] There are few studies on the prevalence of IgAN in Pakistan. Recently published article by Mubarak M is an example which highlights the prevalence of IgAN in Pakistan as a tip of iceberg.[18] It is important to diagnose this entity correctly as it progresses to end stage renal disease in up to 30% cases at 10 years.

Our data showed that two cases diagnosed as MCD on LM turned out to be early stage membranous GN when

IF was performed. Similar findings were seen in two cases initially diagnosed as mesangial expansion/mesangial proliferation on LM. Detection of early stage membranous GN is important, as majority of these cases will be diagnosed as MCD on LM alone. Membranous GN is routinely diagnosed on GMS staining, but early stage may be missed as this can only be appreciated on IF. Treatment includes cyclophosphamide therapy and correct diagnosis changes the prognosis of the disease.[19]

IgM nephropathy is a subset of minimal change disease that may evolve into FSGS. It has higher recurrence rate and poor response to steroids. Detection of IgM nephropathy can be missed if only light microscopy is performed. In our study, IgM nephropathy was diagnosed on IF in 2 cases, the initial morphological diagnosis in these being MCD and Mesangioproliferative glomerulonephritis respectively. The prevalence of IgMN, however, was very low in our study, as compared to other studies.[20]

In our study there were 5 cases of lupus nephritis that were diagnosed on light microscopy on the basis of positive lupus serology. Immunoflorescence plays a key role in diagnosing silent lupus nephritis (SLN). SLN is highly prevalent in renal asymptomatic patients with otherwise systemic lupus erethematosus.[21]

The study had limitations in terms of its sample size calculation. Since glomerulonephritis is not a very common disease, it was difficult to follow routine sample size methodology, but the number — 207 cases — was found worthy enough for our study.

Conclusion

Immunoflorescence along with light microscopy is required for accurately diagnosing renal diseases. This is especially important in cases of early stage membranous, IgAN and IgMN, as these entities cannot be diagnosed with light microscopy alone. Treatment strategy for these cases is aggressive, affecting the progression of the disease.

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