Immunofluorescence Studies of Renal Biopsies

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Immunofluorescence microscopy is the important tool for the diagnosis of glomerular diseases. Direct immunofluorescence (DIF) technique was established and being routinely done at the department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU) since July 2000. This study was taken to diagnose different types of glomerulonephritis by immunofluorescence technique. A total of 327 renal tissues were received for routine and DIF study till 3rd March 2002. Mesangioproliferative glomerulonephritis (112 = 34.3%) topped the list followed by mesangiocapillary glomerulonephritis (66 = 20.2%). Immune deposition was observed in 260 (80%) cases and the predominant deposits were IgM 75 (67%). The pattern of deposition was granular in all cases either in the mesangium and/or along the glomerular basement membrane except one case where linear deposits were observed and diagnosed as membranous glomerulonephritis. Only one case of minimal change diseases showed IgM deposition in the mesangium. Twenty six (7.9%) cases fulfilled the clinical, serological and immunohistopathological criteria of lupus nephritis. Clinically, majority of patients presented as nephrotic syndrome (200 = 61.2%). To obtain a correct diagnosis of glomerulonephritis requires direct immunofluorescence microscopy in parallel with light microscopic examination of renal biopsies and correlation with clinical features, biochemical and serological markers.

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Introduction

Glomerular disorders constitute one of the major causes of morbidity and mortality.[1] Immune mechanisms are responsible for glomerular injury in most cases of primary glomerulonephritis (GN) and many of the secondary GN.[2] Immunofluorescence (IF) microscopy provided insight not only into the pathogenesis of glomerular diseases but also very useful in diagnosing primary renal diseases, assessing the nature and severity of renal involvement in various systemic disorders and in addition, yielded important correlations and prognostic features.[3] Correct diagnosis of glomerulonephritis requires renal biopsy and histopathological examination by light, immunofluorescence and electron microscopic examination, and correlation with clinical features and biochemical parameters.[4] Facilities for electron microscopic study is not readily available in many institutions. In most cases light microscopy (LM) and direct immunofluorescence (DIF) study are more than enough for definitive diagnosis of

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glomerulonephritis.[4] Membranous glomerulonephritis (MGN) stage-1 shows no thickening of the glomerular basement membrane (GBM) and no spikes on silver stain and may not be distinguished from minimal change disease (MCD).[5]

Immunofluorescence showing fine granular deposition of immunoglobulins and complement confirm the diagnosis and distinguish from antiglomerular basement membrane nephritis where liner deposits in DIF and anti-GBM antibody in serum are seen.[5, 6] Lupus nephritic patient of WHO class V also shows immunohistological features of primary MGN and hepatitis B virus related nephropathy. At the time of biopsy, when systemic lupus erythematosus is not clinically suspected, the diagnosis between hepatitis B virus related membranous nephropathy and lupus nephritis may be difficult or impossible to differentiate.[7] Although subendothelial and mesangial prominent IgA deposits are suggestive and only tubular basement membrane deposits are specific, correct diagnosis is made by correlating typical clinical features and serological markers.[5] Hepatitis B infection may be occult, serum transaminase may be normal and there may be no history of clinical hepatitis. The study of glomerular viral antigens and serological screening is important for the diagnosis of hepatitis B virus-related nephritis.[7] Since, glomerulonephritis is a major problem in our country. DIF technique was established and being routinely done at the department of Pathology, BSMMU since July 2000. This study was taken to diagnose different types of glomerulonephritis by immunofluorescence technique in our setting and to show the importance of clinical, biochemical and serological features for correct diagnosis.

Methods

This study was carried out at the department of Pathology BSMMU, Dhaka. A total of 327 renal biopsy specimens were received from July 2000 to March 2002. Two samples of renal tissue were obtained from each patient by percutaneous needle biopsy of clinically suspected GN. Biopsy specimens for LM examination were fixed in 10% formalin and embedded in paraffin. Sections were cut at 4-5 μm thickness from paraffin embedded material and stained routinely with haematoxylin and eosin (H&E), periodic acid-schiff (PAS) stain, and silver stain in some cases. Specimens for DIF microscopy were received in normal saline and immediately frozen and embedded in O. C. T. compound. Cryosectioned at 2-3 μm thickness were done and mounted on glass slides. The slides were air dried and washed in phosphate buffer saline (PBS, pH 7.2) and again air dried. The sections were then stained with fluorescence conjugated anti-sera against human IgM, IgG, IgA, C_3 and fibrinogen, and incubated in moist chamber at room temperature. The sections were washed with PBS and mounted in 90% glycerol in PBS (pH 8.0-9.0). The intensity of fluorescence was graded arbitrarily as none (-), trace (+/-), 1(+), 2(+) and 3(+). Clinical information regarding age, sex, duration of onset of disease, presentation, urine analysis, biochemical and serological parameters were collected from data submitted along with biopsy by the nephrologists.

Results

Of the 327 biopsies obtained from clinically suspected cases of glomerular diseases 184 (56.3%) were male and 143 (43.7%) were female and male female ratio was 1.3:1. Maximum numbers of patients were from 21-30 years age group. Mesangioproliferative glomerulonephritis (112 = 34.3%) topped the list in this study followed by mesangiocapillary glomerulonephritis (66 = 20.2%), membranous glomerulonephritis (35 = 10.7%) and other types of renal disorders (70 = 21.4%) is shown in Table 1. Definitive

diagnosis was not possible in 44 (13.5%) cases because the biopsy represented renal medulla in 32 cases, 10 cases were considered inadequate due to less than five glomeruli, and two adequate cases had diffuse global hyalinized glomeruli.

Of 327 cases, DIF examination was done in 325 cases because tissues were received only for routine examination in two cases. Among 325 cases 260 (80%) were positive and 8 (2.5%) were negative. No glomerulus was seen in 57 cases. Table II shows frequency of immune deposits in common glomerular diseases. Among 7 cases of minimal change diseases one showed mild increased cellularity and matrix in the mesangium by LM and mesangial deposits of IgM in DIF. Others were normal in DIF and light microscopy. Twenty six (7.9%) specimens fulfilled clinical, morphological and immunofluorescence criteria for lupus nephritis, one was male and others were female. All of 26 cases antinuclear antibody (ANA) and anti-DNA antibody were positive. The predominant deposits in DIF of lupus nephritis was C_3 (25 = 96.2%) followed by IgM (22 = 84.6%) and fibrinogen in crescent of one case (Table III). Biopsies from three cases of renal allograft patients found no immune deposits and were diagnosed as acute transplant rejection. Membranous GN was diagnosed in 35 cases. C_3 deposition was found in 74.3% followed by IgG 71.34% (Table II). All of these deposits were fine granular at the GBM as shown in fig.1 except one case where linear deposits of IgG and C3 along the GBM and granular IgM deposits in the mesangium were observed.

Table IV shows the clinical presentation of common glomerulonephritis. The commonest presentation was nephrotic syndrome (200 = 61.2%) followed by proteinuria (54 = 16.5%). Nephritic syndrome was the characteristic presentation of acute diffuse proliferative glomerulonephritis. In 18 (5.5%) cases clinical presentation was not available in the record.

Table1. Diagnosis of 327 patients

Diagnosis	No. of patients	Percentage (%)
Mesangioproliferative GN	112	34.3
Mesangiocapillary GN	66	20.2
Membranous GN	35	10.7
Lupus nephritis	26	7.9
IgA nephropathy	11	3.4
Focal segmental GN	09	2.8
Acute diffuse proliferative GN	08	2.5
Minimal change disease	07	2.1
Focal segmental glomerulosclerosis	06	1.8
Transplant rejection	03	0.9
Undecided	44	13.5

GN = Glomerulonephritis

Table II. DIF findings of glomerulonephritis (%)

Disease	C_3	IgG	IgM	IgA	F
MesPGN (n=112)	65(58.0)	52 (46.4)	75(67.0)	6(5.4)	1(0.9)
MCGN (n=66)	50(75.8)	47(71.2)	46(69.7)	5(7.6)	6(9.1)
MGN (n=35)	26(74.3)	25(71.4)	18(51.4)	1(2.9)	
LN (n=26)	25(96.1)	19(73.1)	22(84.6)	17(65.4)	1(3.9)
IgAN (n=11)	5(45.5)	3(27.3)	4(36.4)	11(100)	
ADPGN (n=08)	6(75)	5(62.5)	6(75)	1(12.5)	
FSGN(n=9)	8(88.9)	2(22.2)	7(77.8)	1(11.1)	1(11.1)
FSGS(n=6)	4(66.7)	1(16.7)	3(50)		
MCD(n=7)			1(100)		

MesPGN = Mesangioproliferative GN, MCGN = Mesangiocapillary GN, MGN = Membranous GN, LN = Lupus Nephritis, IgAN = IgA Nephropathy, ADPGN = Acute diffuse proliferative GN, FSGN = Focal segmental GN, FSGS = Focal segmental glomerulosclerosis, MCD = Minimal change disease, F = Fibrinogen.

Table-III. Immunofluorescent and serological features of lupus nephritis

Total	ANA	Anti-DNA	DIF Findings					WHO-Class				
26	26	26	C3	IgG	IgM	IgA	F	I	II	III	IV	V
			25	19	22	17	1	0	7	2	10	7

F = Fibrinogen

Table IV. Clinical presentation of common glomerulonephritis

Mode of presentation	MesP (n=112)	MC (n=66)	M (n-35)	LN (n=26)	IgAN (n=11)	ADP (n=8)	MCD (n=7)	FSGN (n=9)	FSGS (n=6)
NS (%) (n=200)	72(64.3)	45(68.2)	26(74.3)	10(38.5)	5(45.4)	2(25)	5(71.4)	4(44.4)	6(100)
PROT (%) (n=54)	21(18.7)	3(4.6)	4(11.4)	13(50)	2(18.2)	6(75)	1(14.3)	3(33.3)	
NIS (%) (n=27)	6(5.4)	8(12.1)	3(8.6)	1(3.8)	1(9.1)				
HAEM (%) (n-11)	3(2.7)	4(6.0)		1(3.8)	1(9.1)			2(22.2)	
RF (%) (n=11)	6(5.4)	4(6.0)							
NS & NIS (%) (n=6)	1(0.8)	1(1.5)			2(18.2)				
N/A (%) (n=18)	3(2.7)	1(1.5)	2(5.7)	1(3.8)			1(14.3)		

NS=Nephrotic syndrome, PROT=Proteinuria, NIS=Nephritic syndrome, HAEM=Haematuria, RF=Renal failure, NA=Not available, MesP=Mesangioproliferative GN, MC=Mesangiocapillary GN, M=Membranous GN, LP=Lupus Nephritis, IgAN=IgA Nephropathy, ADP=Acute diffuse proliferative GN, MCD=Minimal change disease, FSGN= Focal segmental GN, FSGS= Focal segmental glomerulosclerosis.

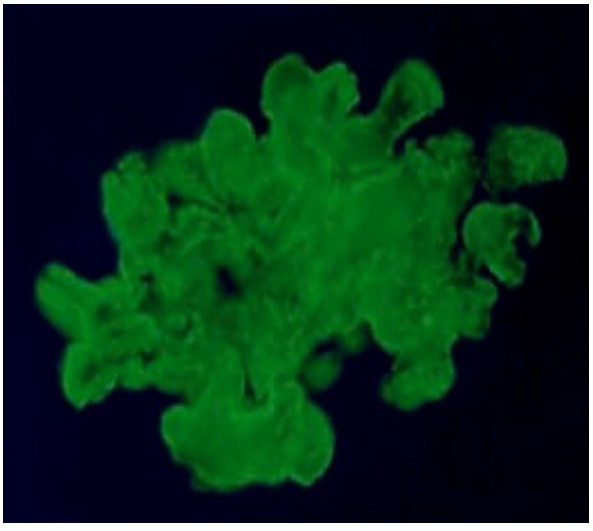

Fig.1. Membranous glomerulonephritis showing granular deposition of IgG along the glomerular basement membrane (DIF stain X 1600)

Discussion

Since glomerulonephritis are immunologically mediated, immunofluorescence microscopy has no alternative for diagnosis of glomerular diseases. Yet, combined analysis of LM and DIF findings and correlation with clinical, biochemical and serological features are essential for accurate diagnosis.

In the study of 327 cases MesPGN (112 = 34.3%) was the most common pattern of glomerulonephritis followed by MCGN. There is a great variation regarding the commonest pattern of glomerulonephritis in different countries. But present study has similarities with many of the studies. In the study of Cheong et al. of Malaysia (1982), Altango et al. of Ethiopia (1982) stated that

proliferative GN was the commonest pattern of glomerulonephritis cited by Rahman.[8] Study of Tabassum, Ahmed and Towhid showed that MesPGN was the commonest pattern of glomerulonephritis in Bangladesh.[1,2,9]

Diagnosis of MCD usually made by absence of glomerular alteration in LM and lack of immune deposits in DIF.[10] Minor degrees of mesangial matrix increase and/or hypercellularity also form an integral part of MCD,[10, 11] but when it is more prominent, it is difficult to segregate from MesPGN.[11] Of the 7 cases of MCD in this study six showed no significant alteration in LM and no immune deposits in DIF. Mild increased mesagial expansion and cellularity with mesagial IgM deposition was observed in one case of previously diagnosed MCD and considered as "IgM associated nephropathy". Since IgM deposits are also seen in MesPGN and focal mesangial sclerosis, whether this represents a separate entity (IgM associated nephropathy) or simply a part of spectrum of MCD-focal sclerosis-MesPGN yet not decided and will require further study.[11, 12] All but one case of idiopathic MGN had linear deposits of IgG and C_3 along the GBM and granular IgM in the mesangium. Linear deposit is the typical character of anti-GBM disease.[11] Anti-GBM disease comprises about 2-5% of glomerulonephritis.[13] Diagnosis depends on detection of sharp, continuous, linear deposition of immunoglobulins at the GBM and demonstration of anti-GBM antibody in the serum.[13] Moorthy et al. (1976) reported three patients with anti-GBM nephritis superimposed on pre-existing MGN cited by Rajaraman.[13] They explained that intermingling of immune complexes with newly formed basement membrane material may alter the antigenecity of basement membrane, leading to the formation of anti-GBM antibody or reverse sequence i.e. superimposition of immune complex disease on anti-GBM nephritis might be the cause. Study of Rajaraman reported six cases of GN with coexistent immune deposits and anti-BM activity.[13] These six patients had documented anti-GBM disease due to presence of liner IgG deposits along the GBM and antibody in the serum. In addition granular deposits of immunoreactants along the GBM or in the mesangium or both observed in immunofluorescence and ultra-structural studies of all six cases. The reason for linear deposition of IgG and C_3 along glomerular basement membrane and IgM deposition in the mesangium observed in our patient could not be identified because anti-GBM antibody and circulating immune complex were not performed. Probably this may be due to superimposition of anti-GBM disease on pre-existing MGN. The predominant immune deposits in MesPGN was IgM in the mesangium and in MCGN was C_3 along the glomerular basement membrane. Similar findings were also observed by other studies.[7, 8, 14, 15]

The most common clinical presentation of the study group was nephrotic syndrome (200 = 61.2%) followed by proteinuria (54 = 16.5%). Nephritic syndrome was the most frequent presentation of ADPGN and nephrotic syndrome was the commonest mode presentation in MGN. The clinical presentation in the present study correlated with the findings of other studies.[1, 2, 8, 9, 10, 16]

Conclusion

Mesangioproliferative glomerulonephritis still remains to be the commonest one in adult patients of glomerulonephritis. Nephrotic syndrome was the predominant clinical presentation in common pattern of glomerular disease. Direct immunofluorescence microscopy and correlation with LM, clinical, biochemical and serological markers should be done on regular basis for correct diagnosis of glomerular diseases.

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