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Immunopathological and Clinical Correlation of Glomerular Diseases: A Light and Immunofluorescence Microscopic Study at Tertiary Care Hospital.

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Abstract

Glomerulonephritis (GN) is a common renal disease usually causing chronic kidney disease especially in developing countries like Bangladesh. Chronic kidney disease (CKD) is a major public health problem increasing worldwide1. In 2017, chronic kidney disease was ranked the eighth highest cause of all deaths2. Diabetes mellitus (46%) is the most common cause of end-stage renal disease followed by hypertension (19%), chronic glomerulonephritis (GN) (15%), and inherited kidney disease (5%)3. Chronic kidney disease accounts for more than one-third of patients of end stage renal disease (ESRD) requiring either dialysis or transplantation. Glomerular diseases are numerous and very difficult to diagnose without a renal biopsy. Renal biopsy is now a days gold standard test throughout the world.

This study aimed to analyze the renal biopsy specimens in a teaching hospital of Bangladesh for proper diagnosis of glomerular diseases. In total, 335 renal biopsies were retrospectively analyzed at National Institute of Kidney Diseases and Urology, Dhaka, Bangladesh for the period of January 2023 to December 2024.

The purpose of this study was to demonstrate the frequency, type, intensity, pattern and site of deposition of immunoglobulin IgG, IgA, IgM and C3 by direct immunofluorescence microscopic technique (DIF) in various pattern of GN and to correlate with clinical and histopathological findings.

Among 335 cases of renal biopsy, 25 cases were discarded due to inadequacy of glomeruli under light microscope and another 10 cases were discarded due absent of glomeruli during DIF study. Finally 300 cases were taken for both histopathologic and direct immunofluorescence microscopic study. In this series, maximum numbers of cases were found in 25-35 age group. Primary glomerular diseases were more common in males (55%) and secondary glomerular diseases more common in females (75.5%). Lupus nephritis (LN) was the most common secondary glomerular disease found in our study. Most frequent clinical presentation and pattern of glomerulonephritis were nephrotic syndrome (60.66%; n=300) and mesangioproliferative GN (41.67%) respectively. Among 300 cases of study group, 149 cases (49.67%; n=300) were DIF positive.

The most frequent single type of immune deposits in various combination was C3 (99.32%, n= 149) followed by IgG (65.8%, 98 out of 149 cases) and IgA (38.81%). The most frequent combination of deposits were IgG+C3 found in 54 cases (36.24%) followed by IgA+C3 combination in 20.01% cases. Mesangium followed by glomerular basement membrane were the most frequent site and granular pattern was the most frequent pattern of deposition.

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Hossain T, et al., J Radiol Clin Imaging 2025
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Deposition of C3 or IgM alone was only 2.69% and 0.68% respectively. Combination of IgM with other antibody was least common (30.81%) whereas IgA combination with others was 38.81%. All of the IgA nephropathy cases were combined deposition of IgA with C3 but not all of the IgA nephropathy were associated with IgG or IgM.

There was a correlation between histopathologic pattern of GN and type-site-pattern of deposition in the glomeruli. Immune-depositions were cent percent in IgA nephropathy, membranous GN (MGN), diffuse proliferative GN and membranoproliferative GN. The most frequent pattern and clinical presentation of GN was mesangioproliferative GN in IgA nephropathy.

In this study, DIF was proved to be essential, sensitive and specific diagnostic tool in the evaluation of glomerular diseases. However, DIF study is of no substitute of light microscopic study but both provide information which when taken as a whole contributes to better understanding of GN.

Keywords: DIF; GN; CKD; ESRD; LN. DIF

Introduction

Glomerulonephritis is the common renal disease causing chronic kidney disease (CKD) speciall in developing countries. To diagnose CKD, renal biopsy remains the "gold standard" technique for histopathologic examination. Light microscopy (LM), immunofluorescence (IF) and electron microscopy (EM) play an essential part in the final diagnosis. To evaluate glomerular diseases, we have to correlate complete clinical and laboratory informations with light, immunofluorescence and electron microscopic findings.

Once, most of the glomerulopathies were evaluated, classified and named according to their histopathologic patterns. A given clinical syndrome can be associated with several histopathologic patterns, conversely, one histopathologic pattern of glomerulonephritis (GN) can be associated with various clinical syndrome. Focal as well as diffuse proliferative GN and crescentic GN usually present with nephritic syndrome [2].

On the other hand, nephrotic syndrome is the typical clinical presentation of the glomerular basement membrane (GBM) disease, visceral epithelial cells with membranous GN, minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS). But membranoproliferative GN (MPGN) presents with combination of nephritic-nephrotic syndrome. Glomerular deposition diseases also presents with nephrotic, nephritic syndrome or combination of both. The sites, types and intensity of antibody deposition causes marked clinical and morphologic overlap. But the site of the antibody deposition within the glomerulus mostly determine the clinico-pathologic presentation [2].

Because anionic antigens are usually repelled by the GBM and trapped in the sub-endothelial and mesangial location causing proliferative GN and relatively cationic antigens tend to deposit in the sub-epithelial aspects of GBM. Visceral epithelial injury causing nephrotic type presentation. Acute sub-endothelial deposits are the causing factor for nephritic syndrome and sub-epithelial, intramembranous immune-deposits for nephrotic range proteinuria [2].

Now a days, direct immunofluorescence microscopic (DIF) study is considered as the advancing modern technique to understand the human glomerular diseases. Therefore, DIF is an urgent need to have a further study for understanding the pattern of GN. By DIF study, one can easily correlate glomerular diseases with light microscopic and clinical findings3. Immune-deposition of IgG, lgA, C3 are awere found to be higher in frequency with patients of haematuria4.Since the treatment of glomerulonephritis is mostly determined by the histopathologic pattern. So, any technique that indicate particular lesion easily, definitely and quickly, might be of clinical importance. Thus, the use of histopatholgy with DIF technique will make GN diagnosis so useful. Therefore, an attempt to correlate DIF technique with the clinical and histopathologic pattern will further enhance the understanding of GN.

In the light of above context, renal biopsy specimens were analyzed to correlate DIF with the various pattern of glomerulonephritis and clinical syndromes.

Materials and Methods

This retrospective study was carried out in the Department of Histopathology, National Institute of Kidney Diseases and Urology, Sher-E-Bangla Nagar, Dhaka during the period of January 2023 to December 2024.Ultrasonographic techniques were used to locate the kidney properly. Proper consent were taken from the selected and admitted patients of National Institute of Kidney Diseases and Urology (NIKDU) for renal biopsy.

A total of 335 renal biopsy (needle biopsy) specimens were selected from the clinically diagnosed cases of nephritic syndrome, nephrotic syndrome, proteinuria or asymptomatic hematuria etc from various age groups. Of the 335 renal biopsied patients, 300 were selected for study because of adequacy of specimens by light microscope and DIF study. Clinical informations were also collected from a pre-designed proforma. Renal biopsies were done by Nephrologist. At least two linear specimens of 10-20 mm in length renal tissues, one in10% formalin for histopathology (light microscopic study) and another one in normal saline for DIF study were taken. Cryostat machine for frozen section to make section in -25 degree Celsius and Fluorescent microscope (NIKON; Labophot-2, model-661012, Japan) with commercially prepared fluorescein isothiocyanate (FITC) conjugated goat antihuman IgG, IgA, IgM, and C3 reagent (Sanofi

Hossain T, et al., J Radiol Clin Imaging 2025
DOI:10.26502/jrci.2809108

Diagnostics Pasteur, Inc. 1000 lake Hazeltine Drive, Chaska, USA) were ready for DIF study. Formalin-fixed processed tissue section were stained by both haematoxylin- and eosin (H & E). Periodic Acid Schiff (PAS) stain was done for GBM thickness study by light microscope. Histopathology and DIF study of the biopsied specimens were performed by the author himself. Presence of at least 5 glomeruli under light microscopic and presence of at least one glomerulus under fluorescent microscope were considered as adequate for study5.

More than 3 mesangial cells embedded in matrix of one segment were considered for Mesangial proliferation and more than 2 nuclei per capillary loops and leukocyte infiltration regarded as endothelial proliferation. More than 5 leukocytes per glomerulus were considered as infiltration6.

At least 50% of the glomerular involvement of crescent formation was crescentic GN. Minimal change disease. When no evidence of any change or presence of minimal mesangial proliferation was taken as minimal change disease (MCD). Focally and segmentally affected glomeruli were considered as focal and segmental proliferative GN and diffusely and globally affected glomeruli as diffuse proliferative GN [7]. Predominant mesangial deposition of IgA along with C3 detected by immunofluorescence microscopy with history of hematuria was diagnosed as IgA nephropathy8.

Clinical presentation and patterns of IgA nephropathy in this study were evaluated separately. Segmental sclerosis of one or two lobules with hyalinosis involving portions of fewer than 50% of the glomeruli in a section was regarded as Focal segmental glomerulosclerosis (FSGS). Increase in amount of homogeneous non-fibrillar extracellular material of similar composition to GBM and mesangium was defined as sclerosis Membranous GN: Diffuse thickening of GBM due to sub-epithelial deposits of immune complex without evidence of inflammation or cellular proliferation.

Membranoproliferative GN: Diffuse thickening of GBM with predominant proliferation of mesangial cells and extension of matrix often with interposition in between the endothelial cells and GBM causing tram-track appearance under light microscope [9].

Mesangioproliferative GN: Diffuse increase in glomerular cellularity predominantly due to mesangial cells often with concomitant increase in mesangial matrix [9]. In both cases of light microscopic and DIF study, photographs were taken.

Fluorescein dye conjugated antihuman antibodies (lgG, IgA, IgM and C3) were fixed by tissue section of ten times diluted anti-sera. Fall of ultraviolet light into the stained tissue section were done. It made excitation and emission of light of higher wave length. Deposition was visible (apple green colour) under fluorescent microscope if there is any antigen in the tissue section. Here antibodies and complement lies within the tissue section act as antigen and artificially prepared antihuman antibodies-complement as antibody. Total number of glomeruli, GBM, mesangial, endothelial and epithelial cells and infiltration of inflammatory infiltrates, interstitium, blood vessels and tubules were observed under lightmicroscope6,7. Pattern (granular or linear), type (lgG, IgA, IgM and C3), sites and intensity of deposition in the glomeruli were observed under fluorescent microscope. FITC staining intensity was graded from 0 to +3. Zero (0) being negative and +3 was maximum intensity (mild +1; moderate ++2; marked +++3)6.7 . Histopathological diagnosis of formalin-fixed processed renal tissue were made based on H&E and PAS stain.

Results

Of the 335 renal biopsy cases, 25 cases were labeled as inadequate for histopathologic study and 10 cases showed no glomeruli and regarded as inadequate for DIF study. Relevant clinical, histopathological and DIF study were performed on the remaining 300 cases. Of 300 study cases, 165 were male (55%) and 135 (45%) were female. The age ranged from 6-65 years and mean age of 32.67 years.

Table-1 shows that the most frequent pattern of GN was Mesangioproliferative GN (41.67%; n=300) followed by focal segmental proliferative GN (21%), Minimal change disease (MCD,12%), Mebrenoproliferative GN (10%), Membranous GN (05%), Crescentic GN (04%), Focal segmental glomerulosclerosis (03%), Lupus nephritis (1.66%) and Diffuse proliferative glomerulonephritis (0.67%) respectively among all age group. The most frequent clinical presentation of the study group was the nephrotic syndrome (60.66%) followed by nephritic syndrome (12.67%) and haematuria (11%), CKD (7%), proteinuria (6.67%) and in the form of RPGN (02%).

Of the clinically presented with nephrotic syndrome, most frequent histopathologic pattern were mesangioproliferative GN (26%, n=300) followed by focal segmental proliferative glomerulonephritis and minimal change disease (12% each), membrenous glomerulonephritis (05%),membranoproliferative glomerulonephritis (03%), lupus nephritis (1.66%) and focal segmental glomerulosclerosis (01%).

In this study group, 12.67% showed clinical presentation with nephritic syndrome. Of the nephritic syndrome, histopathologic pattern were mesangioproliferative glomerulonephritis (05%, n=300), focal segmental proliferative glomerulonephritis (04%, focal segmental glomerulosclerosis (02%), membrenoproliferative glomerulonephritis (01%) and diffuse proliferative glomerulonephritis (0.67%).

Out of 300 hundred selected cases for study, 33 cases (11%) showed hematuria clinically. Of the 33 (11%) cases of

Hossain T, et al., J Radiol Clin Imaging 2025
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Table 1: Clinical presentation and histopathological pattern of (n=300)

Clinical Features	Pattern of Glomerulonephritis (n=300)									
	MSPGN	FSPGN	MCD	MPGN	MGN	Crescent	FSGS	LN	DGN	Total
	125 (41.67%)	66	36	30	15	12	9	5	2	(%)
		-22%	-12%	-10%	-5%	-4%	-3%	-1.66%	-0.67%	
Nephrotic syndrome	26	12	12	3	5	-	1	1.66	-	60.66
Nephritic syndrome	5	4	-	1	-	-	2	-	0.67	12.67
Hematuria	5	2	-	2	-	2	-	-	-	11
CKD	2	3	-	2	-	-	-	-	-	7
Proteinuria	3.67	1		2	-	-	-	-	-	6.67
RPGN	-	-	-	-	-	2	-	-	-	2
Total (%)	41.67	22	12	10	5	4	3	1.66	0.67	100

MSPGN: Mesangioproliferative glomerulonephritis

FSPGN: Focal segmental glomerulonephritis; MCD: Minimal change disease

MGN: membranous glomerulonephritis MPGN: Membrenoproliferative Glomulonephritis; GSGS: Focal segmental glomerulonephritis

DGN: Diffuse proliferative glomerulonephritis; RPGN: Rapidly progressive glomerulonephritis. Lupus nephritis: LN

hematuria, 27 cases (9%) showed hematuria with deposition of IgA and C3 mostly in the mesangial region. So, these 27 cases of hematuria (9%) regarded as IgA nephropathy. Rest 6 cases (2%) of hematuria were associated with other pattern of glomerulonephritis without any deposition in the glomeruli. Of the IgA nephropathy of 27 cases (9%, n=300), 5% showed histopathologic pattern of mesangioproliferative GN,2% focal segmental proliferative GN and 2% as membrenoproliferative GN.

In this study, Clinical diagnosis of chronic kidney disease (CKD) were 7% of the total 300 study group of which showed 02% mesangioproliferative GN, 03% focal segmental proliferative GN and 02% membranoproliferative glomerulonephritis histopathologically. This study represents the clinical features of proteinuria about 6.67% of which showed 3.67% mesangioproliferative GN, 02% membrenoproliferative GN and rest 01% as focal segmental proliferative GN under light microscope.

Rapidly progressive glomerulonephritis (RPGN) is the clinic-pathologic counterpart of crescentic glomerulonephritis which represents only 02% of the study group. This 02% of RPGN were also diagnosed as crescentic glomerulonephritis under light microscope. Another 02% of total 04% crescentic glomerulonephritis showed clinical presentation of hematuria in the study.

DIF study was performed in 300 cases of which 149 cases (49.67%) were DIF positive and another 151 cases (51.33%) DIF negative (Table- 2).

Table 2: Histopathological pattern and DIF findings (n=300)

Pattern	Number of cases	DIF positive Cases	DIF positive (%)	DIF negative cases	DIF negative (%)
MSPGN	125 (41.67%)	66	52.8	59	47.2
FSPGN	66 (22%)	16	24.24	50	75.76
MCD	36 (12%)	6	16.67	30	83.33
MPGN	30 (10%)	30	100	0	0
MGN	15 (5%)	15	100	0	0
Crescentic	12 (4%)	6	50	6	50
FSGS	9 (3%)	3	33.33	6	66.67
Lupus neph.	5 (1.66%)	5	100	0	0
DPGN	2 (0.67%)	2	100	0	0
Total	300 (100%)	149	49.67%	151	51.33%

Hossain T, et al., J Radiol Clin Imaging 2025
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The sites of deposition of IgG, IgM, IgA and C3 were predominantly in the mesangium in case of IgA nephropathy, mesangioproliferative GN, FSGS and focal segmental proliferative GN. Antibody deposition were predominantly in the GBM in case of membranous and membranoproliferative GN. Sub-endothelial deposition were predominant in diffuse proliferative GN. In crescentic glomerulonephritis, linear pattern of deposition of antibodies were typically observed in the glomerular basement membrane (GBM) along the glomerular capillary walls under fluorescent microscope. DIF positivity were observed in all IgA nephropathy, membranoproliferative GN, MGN, MPGN, DPGN and lupus nephritis in the present study. In case of MSPGN, MCD, FSGS, FSPGN and crescentic GN, deposition were (52.80%, n=149) 16.67%, 33.33%, 24.24% and 50% respectively (Table-2).

The most frequent single type of immune deposits in various combination was C3 (99.32%, n= 149) followed by IgG (65.8%, 98 out of 149 cases and IgA (38.81%). The most frequent combination of deposits were IgG+C3 found in 54 cases (36.24%) followed by IgA+C3 combination in 20.01% cases. Deposition of C3 or IgM alone was only 2.69% and 0.68% respectively. Deposition combination of C3 with others was the most common (99.32%) and combination of IgM with other antibody was least common (30.81%) whereas IgA combination with others was 38.81%. All of the IgA nephropathy cases were combined deposition of IgA with C3 but not all of the IgA nephropathy were associated with IgG or IgM shown in this study.

There is a great variation of DIF positivity by different authors in home and Abroad [7,24,4,13,14,15].

Table 3: Distribution of Immunoglobulin IgG, IgA, IgM and C3 of 149 (49.67%) DIF positive cases in various pattern of GN

Immunoiglobulins and C_3	Number of positive cases (n=149)	Percentage (%)
IgG+ C_3	54	36.24
IgA+ C_3	30	20.01
IgG+IgM+ C_3	16	10.76
IgA+IgG+ C_3	14	9.4
IgG+IgA+IgM+ C_3	14	9.4
IgM+ C_3	11	7.4
IgA+IgM+ C_3	5	3.37
C_3 alone	4	2.69
IgM alone	1	0.68
Total	149	100

Table 4: Comparative study of DIF positive cases by different authors

	DIF positive findings in percentage						
Pattern of glomerulonephritis	**Morel-Maroger, 1972**	**Larsen, 1978**	**Metha et al, 1983**	**Tabassum, 1988**	**Sharmin, 1994**	**Nabir, 1996**	**This study, 2024**
MSPGN	53.33	85	45	80	70	60	52.8
FSPGN	74.58	90.57	65	60	50	40	24.24
MCD	-	60	57.1	-	-	-	16.67
MGN	100	100	100	100	100	100	100
MPGN	100	100	100	100	100	100	100
IgA Nephropathy	100	100	100	100	100	100	100
DPGN	83.88	77.7	100	100	100	100	100
Crescentic	76	65	33	100	100	--	100
FSGS	76.32	40	60	33.33	10	-	33.33
Lupus neohritis	100	100	100	100	100	100	100

Hossain T, et al., J Radiol Clin Imaging 2025
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A comparative study with different patterns of DIF positivity in the present study compared with other studies shown in table -4.

Discussion

Both primary and secondary renal glomerular diseases are usually diagnosed by histopathology with special stains. Along with Histopathological examination it also need direct immunofluorescence microscopic (DIF) study. Sometimes Electron microscopic study may be required in selected cases. In the present study 300 cases of glomerular diseases were selected. The clinical and DIF study were correlated with various types of morphological pattern of glomerulonephritis.

In the present study, the maximum number of cases occurred in the 25-35 age group followed by 36-45 age groups. The lowest number of cases occur in 6-25 age groups which more or less similar to most of other studies. Nephrotic syndrome was the most frequent clinical presentation shown by Cameron, 1980 (57.77%) 100. Ziauddin et ai, 1993 (56.6%)11 and Rahman et ai, 1984 (94%)12 similar to our study (61.22%). There was a wide variation regarding the commonest pattern of glomerulonephritis yet done in home and abroad.

In the study of Rahman et al in 1984, Ziauddin et al in1993 and Nabir et al in 1996 showed that MCD, DPGN and FSPGN were the most frequent pattern of GN respectively13. But the study of Tabassum, 1988 and Sharmin, 1994 along with our study reveal mesangio proliferative GN is the most frequent pattern of GN [14,15]. On the other hand, diffuse proliferative GN is the commonest pattern of GN in western world [16,17, 18]. MCD and FSPGN differentiation is very tough by light microscope. It needs serial sections with more number of glomeruli during microscopic study to differentiate MCD from focal segmental proliferative GN.

In fact, mesangial cellularity in cases MCD is an intermediate step for the evolution of MCD to FSGS19. Evidence of segmental proliferations sometimes may be missed due to insufficient number of glomeruli in the the biopsy specimen. Clinico-pathologic correlation of different patterns of GN in this study group was more or less similar to other studies [2].

In this study, DIF was positive in 149 cases (49.67%) and negative in another 151 cases (51.33%). More or less similar results were found by other two investigators in Bangladesh [14,15].

Our study showed predominantly generalized distribution of deposits either in the mesangium or GBM (93.88%). More or less similar findings were obtained by other authors [14,15,20,21]. Usually, apparently normal glomeruli or minimal change under light microscope and no deposition in DIF study regarded as MCD. But in our study, reveals DIF positivity of 16.67% of MCD cases which were similar to a few other studies [4, 21]. Brenner and Rector in 2004 explained regarding DIF positivity due to differentiation between mild prominences of mesangial.cells as.observed in MCD and definite mesanglal proliferation are subjective and highly susceptible to artifacts of sectioning and specimen preparation5. The most frequent type of single immune-deposits in various combinations was IgG followed by C3 and IgA in the study of Tabassum, 1988 and Nabir, 1996 which was dissimilar to our study. Our study showed most frequent deposition was C3 followed by IgG and IgA. The most frequent combination of deposition was lgA+C3 of our present study in IgA nephropathy. This is very similar result reported by Sharmin, 1994 and Khan et ai,1990 [15,23]. It was also observed that the type, site, pattern and intensity of immune-depositions greatly modify the clinical presentation and prognosis of GN.

Conclusion

Renal biopsy become the gold standard technique in the diagnosis of glomerular diseases. Although somewhat expensive, DIF was proved to be simple, sensitive, rapid and highly specific diagnostic tool. Commonest site of deposition was mostly in the mesangium followed by glomerular basement membrane with granular pattern. Various combination of C3 deposition was predominant in our study. Although the site, type, pattern and intensity of immune deposits can be determine by DIF technique. But the exact location of immune deposits not possible in the absence of electron microscope.

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