Section: Pathology

Original Research Article

INTEGRATED EVALUATION OF NEPHROTIC SYNDROME: UTILITY OF IMMUNOFLUORESCENCE, HISTOMORPHOLOGY, AND RENAL PARAMETERS

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ABSTRACT

Background: The aim is to evaluate the clinicopathological spectrum of glomerular diseases in patients with nephrotic syndrome and renal dysfunction and to determine the diagnostic utility of histomorphology, direct immunofluorescence (DIF), and renal parameters in renal biopsy evaluation.
Materials and Methods: A prospective observational study was conducted over 18 months (November 2022–May 2024) at a tertiary care center in South India. Forty native renal biopsies from patients with renal dysfunction were included. Biopsy specimens were examined using light microscopy with hematoxylin and eosin, periodic acid–Schiff, Gomori methenamine silver, and Masson's trichrome stains, followed by direct immunofluorescence. Histopathological and immunofluorescence findings were correlated with clinical presentation and laboratory parameters to establish the final diagnosis.
Result: The study included 21 males and 19 females aged 13–68 years. Nephrotic syndrome was the most common indication for renal biopsy, while hypertension was the predominant associated comorbidity. Membranoproliferative glomerulonephritis was the most common primary glomerular disease, whereas mesangioproliferative glomerulonephritis secondary to lupus nephritis was the leading secondary glomerular disease. Direct immunofluorescence was indispensable for the accurate classification of immune-mediated glomerular disorders, and the use of all eight basic immunofluorescence markers significantly enhanced diagnostic accuracy when interpreted alongside histopathological and clinical findings[1].
Conclusion: Renal biopsy, integrating histomorphology and direct immunofluorescence, remains the gold standard for the diagnosis and classification of glomerular diseases[2]. A combined clinicopathological approach improves diagnostic precision, facilitates disease subtyping, and supports appropriate therapeutic decision-making in patients with nephrotic syndrome.

INTRODUCTION

Nephrotic syndrome, a heterogeneous group of glomerular disorders characterized by heavy proteinuria, hypoalbuminemia, edema, and dyslipidemia, remains a major contributor to chronic kidney disease, particularly in India and other Asian countries where the burden of renal disease is substantial. Primary nephrotic syndrome is most commonly attributed to Minimal Change Disease, followed by Membranoproliferative Glomerulonephritis, whereas Chronic Glomerulonephritis and Diabetic Nephropathy predominate among secondary causes.[1,2]

Accurate diagnosis requires an integrated approach combining histomorphological evaluation, immunofluorescence microscopy, and assessment of renal parameters. Immunofluorescence identifies the pattern and distribution of immune complex deposition, providing valuable insights into the underlying immunopathogenesis of glomerular injury. Histomorphological examination complements these findings by defining structural alterations, while renal biochemical parameters assist in disease classification, severity assessment, and prognostication.[3]

Although regional variations in the spectrum of glomerular diseases have been extensively

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documented, data from the central region of Tamil Nadu, particularly Thiruchirappalli District, remain limited. This study aims to evaluate the clinicopathological spectrum of glomerular diseases in patients with renal dysfunction and compare the observed prevalence and histopathological patterns with national and international reports.

MATERIALS AND METHODS

This prospective observational study was conducted over an 18-month period (November 2022 to May 2024) at Mahatma Gandhi Memorial Government Hospital, Thiruchirappalli. A total of 40 native renal biopsies obtained from patients with renal dysfunction were included. Renal dysfunction was defined by one or more of the following criteria: serum creatinine >1.5 mg/dL, proteinuria >1 g/24 hours (including nephrotic-range proteinuria >3.5 g/24 hours), or microscopic/macroscopic hematuria. Renal transplant biopsies were excluded from the study. Demographic details, clinical presentation, comorbidities, laboratory investigations, and the underlying causes of nephrotic syndrome were systematically recorded using a structured data collection proforma. Renal biopsy specimens were obtained as two cortical tissue cores whenever feasible, with one core fixed in 10% neutral buffered formalin for routine histopathological evaluation and the other preserved in normal saline or Michel's transport medium for immunofluorescence studies. In instances where only a single core was available, it was initially processed for immunofluorescence on frozen sections, following which the remaining tissue was fixed in formalin for light microscopic examination. Histopathological assessment was performed using hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), Gomori methenamine silver (GMS), and Masson's trichrome (MTS) stains[4,5]. Immunofluorescence analysis was carried out to detect glomerular immune deposits. The combined histomorphological and immunofluorescence findings were correlated with the clinical and laboratory data to classify and subtype the spectrum of nephrotic syndrome.

RESULTS

40 renal biopsies were received over a period of 18 months from November 2022 to May 2024.

Nephrotic syndrome was determined by

- Massive proteinuria > 3.5 g/24 hours.
- Hypoalbuminemia < 3 gm/dl.
- Generalized edema.

All transplant biopsies were excluded from the final analysis.

The following parameters were analysed from the cases.

Demographic Data:

[Table 1] Age distribution

Age distribution of study participants is given in [Table 1 and Figure 1]. The mean age ± SD of patients with nephrotic syndrome in the present study was 36.9± 5.0 years with minimum and maximum age of 13 and 68 years respectively. Majority belonged to the age group of 11 to 20 years and 51 to 60 years.

Table 1: Age distribution of patients undergoing renal biopsy (n=40)

Age group (years)	Number of patients	Percentage
11-20	11	27.5
21-30	6	15.0
31-40	6	15.0
41-50	5	12.5
51-60	11	27.5
61-70	1	2.5

(Minimum age- 13, Maximum-68, Mean age ± SD- 36.9± 5.0)

[Table 2] Sex distribution. Around 52% were male and 48% female with nephrotic syndrome in the present study [Figure and Table 3]

Table 2: Sex distribution of patients undergoing renal biopsy (n=40)

Sex	Number of patients	Percentage
Male	21	52%
Female	19	48%

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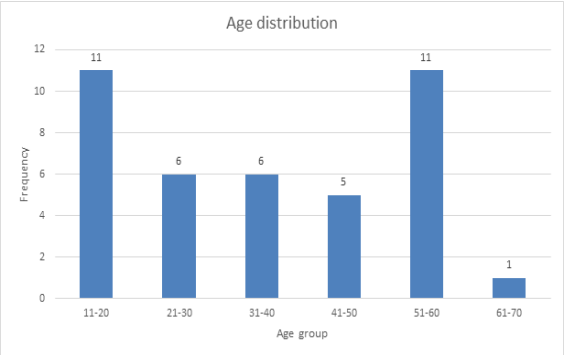

Figure 1: Age distribution of study participants (n=40)

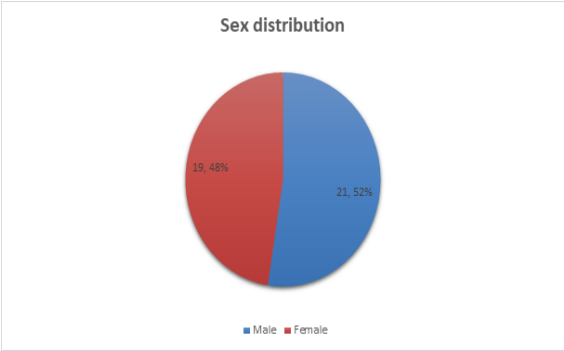

Figure 2: Sex distribution of study participants (n= 40)

[Table 3] Lab parameters. Data of serum creatinine, protein creatinine ratio, proteinuria and hematuria of patients were collected. Details of investigation is given in [Table 3].

Table 3: Laboratory investigation of study participants (n=40)

Investigation		No. of patients	Percentage
Serum creatinine			
Female (n=19)	0.5-1.1	10	52.6
	>1.1	9	47.3
Male (n=21)	0.7-1.3	10	47.6
	>1.3	11	52.4
Protein Creatinine Ratio (n=35)			
<0.2		4	11.4
≥0.2		31	88.5
Not Available		5	-
Proteinuria			
Traces		8	20.0
1+		5	12.5
2+		17	42.5
3+		9	22.5
4+		1	2.5
Hematuria (n=37)			
Present		7	18.9
Absent		30	81.1
Unknowm		3	-

Mean Serum creatinine for Female was 1.25 ± 0.32and Male was 4.39 ± 0.43

[Table 4] Immunofluorescent positivity

Immunofluorescent marker positivity for IgM, IgG, IgA, C3c, C1q, Lambda and Kappa was tested among all samples except one. IgM marker was found to be positive among 25% and 50% of cases of FSGS and MPGN respectively, while it was negative for all others cases. IgG was positive in 100% case of IRGN, MGN and NGS. 71.4% and 66.7% cases of MPGN and EPGN respectively positive for IgG. IgA was reported positive among 100%, 75% and 16.6% of IRGN, MPGN and CGN respectively. C3c was detected only among MPGN (71.4%), EPGN (66.7%) and MPGN (50%). C1q was detected only among 2 cases of MPGN out of 8 (25%). 1 case of MPGN was positive for both Lambda and kappa. Pyelonephritis and normal morphology on immunofluorescent had zero marker positive.

Table 4: Frequency of Immunofluorescence positivity in various renal disease

Renal disease	Immunofluorescent marker positivity					
	IgM	IgG	IgA	C3c	C1q	Lambda/kappa
Crescentic Glomerulonephritis (n=6)	-	1 (16.6%)	1 (16.6%)	-	-	-
Endocapillary Proliferative Glomerulonephritis (n=3)	-	2 (66.7%)	-	2 (66.7%)	-	-
Focal Segmental Glomerulosclerosis (n=4)	1 (25%)	2 (50%)	-	-	-	-
Infection Related Glomerulonephritis (n=1)	-	1 (100%)	1 (100%)	-	-	-
Membranoproliferative Glomerulonephritis (n=7)	-	5 (71.4%)	-	5 (71.4%)	-	-
Membranous Glomerulonphritis (n=3)	-	3 (100%)	-	-	-	-
Mesangioproliferative Glomerulonephritis (n=8)	4 (50%)	4 (50%)	6 (75%)	4 (50%)	2 (25%)	1/1 (12.5%)
Nodular Glomerulosclerosis (n=2)	-	2 (100%)	-	-	-	-
Pyelonephritis (n=1)	-	-	-	-	-	-
Normal Morphology (n=4)	-	-	-	-	-	-

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[Table 5] Utility of immunofluorescence over histopathology. Most of the histopathological diagnosis was same as immunoflurescence except for 4 cases of normal morphology on histopathology diagnosis was found to be FSGS.

Table 5: The Utility of Immunofluorescence on Histopathological Evaluation for the improved diagnostic accuracy

		Immunofluorescence diagnosis										
		PICG	**IgAN**	**Anti GBM**	**IRGN**	**FSGS**	**MGN**	**MPGN**	**LN**	**MSPGN**	**MIDD**	**MCD**
Histopathological diagnosis	Crescentic Glomerulonephritis (n=6)	4	1	1								
	Endocapillary Proliferative Glomerulonephritis (n=3)				3							
	Focal Segmental Glomerulosclerosis (n=4)					Tip variant -2 Perihi; ar variant -1						
	Infection Related Glomerulonephritis (n=1)				1							
	Membranoproliferative Glomerulonephritis (n=7)							7				
	Membranous Glomerulonphritis (n=3)						3					
	MesangioproliferativeGlomerulonephritis (n=8)		2						5	1		
	Nodular Glomerulosclerosis (n=2)							1			1	
	Pyelonephritis (n=1)				1							
	Normal Morphology (n=4)					4						
	Unknown (n=1)					1						

PICG – Pauci Immune cresentric glomerulonephritis
IgAN – IgA Nephropathy
Anti GBM – Anti glomerular basement membrane
IRGN – Infection related glomerulonephritis
FSGS – Focal Segmental Glomerulonephritis
MGN – Membranous glomerulonephritis
MPGN – Membranoproliferative glomerulonephritis
LN – Lupus Nephritis
MSPGN – Mesangioproliferative glomerulonephritis
MIDD – Monoclonal Immunoglobulin deposition disease
MCD - Minimal change disease

Summary of Findings: A total of 40 patients with renal dysfunction underwent renal biopsy, and the specimens were evaluated using histopathological examination and direct immunofluorescence. The study population comprised 21 males and 19 females, with ages ranging from 13 to 68 years. Nephrotic syndrome was the most common clinical presentation, and hypertension was observed in the majority of patients at the time of biopsy. Among primary glomerular diseases, membranoproliferative glomerulonephritis was the predominant histopathological diagnosis, whereas mesangioproliferative glomerulonephritis secondary to lupus nephritis was the leading cause of secondary glomerulonephritis. Direct immunofluorescence proved to be pivotal in achieving an accurate diagnosis of glomerular diseases. Furthermore, the application of all eight basic immunofluorescence markers was essential for establishing a definitive diagnosis, and correlation with urinalysis constituted an indispensable component of the overall diagnostic evaluation.

DISCUSSION

A total of 40 renal biopsies performed for renal dysfunction were analyzed in this study. The study population comprised 21 males (52%) and 19 females (48%), demonstrating a slight male predominance comparable to previous Indian studies. The patients ranged from 13 to 68 years of age, with a mean age of 36 ± 5 years. Nephrotic syndrome was the most frequent indication for renal biopsy, consistent with published Indian literature.
Renal biopsy remains the gold standard for diagnosing glomerular diseases, as systematic evaluation of the glomeruli, tubules, interstitium, and vessels provides essential information regarding the pattern and severity of renal injury. Histopathological examination enables identification of structural alterations such as cellular proliferation, sclerosis, basement membrane abnormalities, tubular injury, interstitial inflammation, fibrosis, and vascular changes, thereby facilitating accurate disease classification.
Among primary glomerular diseases, membranoproliferative glomerulonephritis was the predominant diagnosis in the present study6. This finding differs from reports from other regions of South India, where membranous nephropathy has been more frequently observed, and from studies in Northern and Central India that reported IgA nephropathy and membranous nephropathy as the leading primary glomerulopathies. Among secondary glomerular diseases,

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mesangioproliferative glomerulonephritis secondary to lupus nephritis was the most common diagnosis, which is comparable with previously published data.[9,10]

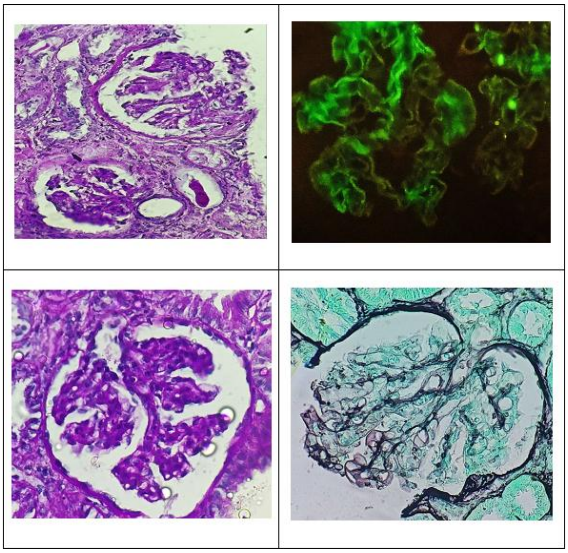

Image 1: Membranoproliferative glomerulonephritis (MPGN). H&E, Immunoflorescence showing IgG granular deposits, PAS staining, Silver staining showing double contour of glomerular basement membrane.

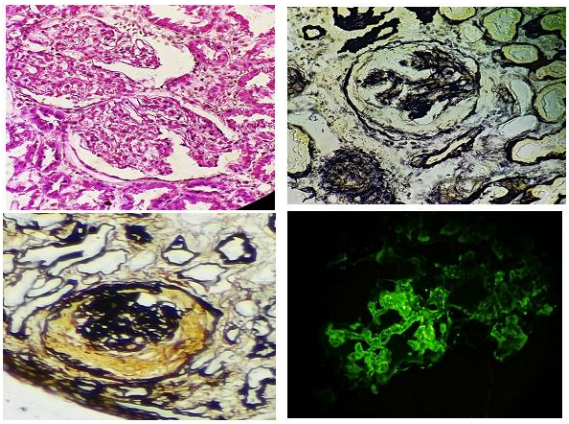

Image 2: Lupus Nephritis. H&E – wire loop lesion, silver staining showing cresent formation, immunofluorescence showing IgG linear positivity along glomerular basement membrane.

Direct immunofluorescence played a crucial role in the accurate classification of glomerular diseases by demonstrating characteristic immune deposits that cannot be reliably identified by light microscopy alone. It was particularly valuable in diagnosing entities such as IgA nephropathy, C1q nephropathy, IgM nephropathy, anti-glomerular basement membrane glomerulonephritis, and immune complex-mediated lupus nephritis7,8. Correlation of histopathological findings with immunofluorescence and clinical parameters significantly improved diagnostic precision.

The use of a complete panel of eight immunofluorescence markers is essential for comprehensive evaluation of renal biopsies. In selected cases, immunofluorescence findings, including light-chain restriction, may identify monoclonal immunoglobulin deposition disease and prompt further evaluation for underlying plasma cell dyscrasias such as multiple myeloma. Therefore, the combined interpretation of light microscopy, direct immunofluorescence, and clinicopathological correlation remains indispensable for accurate diagnosis, prognostication, and appropriate therapeutic decision-making in glomerular diseases.

CONCLUSION

Renal biopsy remains the gold standard for the diagnosis and classification of glomerular diseases. Although light microscopy and special stains provide valuable morphological information, direct immunofluorescence is indispensable for the accurate characterization of immune-mediated glomerular disorders and significantly enhances diagnostic precision. Given the limited availability of electron microscopy, direct immunofluorescence serves as the most practical and cost-effective adjunct in routine renal pathology.

In this study, nephrotic syndrome was the most common indication for renal biopsy, while membranoproliferative glomerulonephritis and mesangioproliferative glomerulonephritis secondary to lupus nephritis were the predominant primary and secondary glomerular diseases, respectively. These findings highlight the importance of an integrated histopathological and immunofluorescence-based approach for accurate diagnosis and optimal patient management.

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