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DIAGNOSTIC YIELD OF DIRECT IMMUNOFLUORESCENCE IN RENAL PATHOLOGY: A CROSS-SECTIONAL STUDY

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ABSTRACT: -

Introduction:Glomerulonephritis (GN) is a common kidney condition and a primary factor in chronic kidney failure, accounting for more than 1/3rd of end-stage renal disease instances that necessitate dialysis or kidney transplants. Direct immunofluorescence (DIF) is a cornerstone technique in renal pathology, crucial for diagnosing various glomerular diseases by detecting immune complex depositions. This study sought to evaluate the diagnostic utility of direct immunofluorescence microscopy compared to histopathology for the diagnosing renal conditions.

Methods: A cross-sectional analytical study was directed on native kidney biopsies received in the Department of Pathology at a tertiary care hospital. Frequency, class, staining intensity, distribution pattern, and localization of immune deposits detected by DIF microscopy across various histopathological categories of glomerulonephritis were analyzed.

Results: Out of a total of 89 renal biopsy cases, 65 cases (73%) were deemed suitable for both histopathological examination and DIF microscopy. Nephrotic syndrome was the predominant clinical manifestation, seen in 63.1% of instances, and focal segmental glomerulosclerosis emerged as the most frequent histologic pattern, comprising 27.7% of cases. Among the 65 adequate samples, DIF yielded positive findings in 38 cases (58.5%). The most commonly detected immune deposits involved C3, in combination, present in 94.7% of cases, followed by IgG (68.4%) and IgA (47.4%).

Conclusion: DIF enhances diagnostic accuracy in renal pathology. Its integration with histopathologic findings remains essential for comprehensive interpretation. Inclusion of DIF in native kidney biopsies is justified by its high diagnostic yield.

INTRODUCTION: -

Glomerulonephritis (GN) describes a variety of conditions marked by inflammation of the glomeruli, often presenting with hypertension, hematuria, edema, and acute kidney damage(1). It can present as various clinical forms, including nephrotic syndrome, nephritic syndrome, asymptomatic hematuria or proteinuria, and rapidly progressive renal failure. GN can be either primary (limited to the kidney) or secondary to systemic conditions like vasculitis, lupus, or infections.

Glomerulonephritis remains a significant cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) globally(2). Accurately quantifying the global burden of GN is challenging because of differences in diagnostic capabilities and practices in renal biopsy. However, data from the Global Burden of Disease Study suggest that GN contributes to approximately 10% - 20% of all ESRD cases worldwide(3).

Accurate diagnosis and classification of GN are crucial, as they directly influence treatment strategies and prognosis. Renal biopsy, the benchmark for the evaluation of GN, offers vital insights into

the underlying pathological processes(4). Histopathological examination through light microscopy provides information on glomerular architecture and the pattern of injury. Direct Immunofluorescence (DIF) microscopy serves as an indispensable adjunct to conventional histology in the diagnostic workup of GN. Without DIF, differentiation between similar-appearing patterns - such as distinguishing post-infectious GN from C3 glomerulopathy can be challenging(5). Consequently, light microscopy by itself might not adequately characterize the immune-mediated mechanisms involved in glomerular damage.

DIF involves the application of fluorescein-labeled antibodies to frozen kidney tissue sections, allowing for the detection and localization of immunoglobulins (IgG, IgA, IgM) and complement components (such as C3) within the glomeruli(6,7). The pattern, intensity, and composition of immune deposits revealed through DIF are critical for differentiating among various forms of glomerulonephritis. The integration of DIF with histopathologic examination enhances diagnostic accuracy, facilitates disease classification, and aids in guiding appropriate management and prognosis.

This study intended to evaluate the histopathological patterns of glomerulonephritis and highlight the diagnostic value of direct immunofluorescence in renal biopsy interpretation.

MATERIALS AND METHODS: -

This cross-sectional analytical research was adopted and data collected over a 1 year period at our Department of Pathology at Saveetha Medical College and Hospital. Institutional Ethics Committee approval was accomplished. Renal biopsy samples were acquired from patients with clinically suspicious and biopsy-recommended glomerulonephritis and analyzed(8). Out of a total of 89 renal biopsy specimens received, 65 cases (73%) were deemed suitable for both histopathological examination and DIF microscopy.

Demographic details and clinical history were retrieved from the archived database and recorded in a structured pro forma. Histopathological diagnosis of formalin-fixed, processed renal tissue examination was conducted through Hematoxylin and Eosin (H&E) and Periodic Acid–Schiff (PAS) staining followed by direct immunofluorescence (DIF) analysis on saline-fixed tissue frozen section beneath -25ºC temperature.

Fluorescein dye-labeled anti-human antibodies (IgG, IgA, IgM, and C3) were attached to the tissue section using tenfold diluted antisera(9). Under ultraviolet light, fluorescent dye in the stained tissue emits an apple-green glow; if specific antigens are present. In DIF, tissue-bound antibodies and complement act as antigens, while fluorescent-labeled anti-human antibodies detect them, revealing immune deposits under a fluorescence microscope.

Under light microscopy, detailed examination was carried out to assess the total number of glomeruli, the Glomerular Basement Membrane (GBM), mesangial, endothelial, and epithelial cells, as well as the presence of inflammatory infiltrates. Direct immunofluorescence microscopy was used to delineate the type of immune deposits (IgG, IgA, IgM, and C3), their archetype, anatomical location, and staining magnitude. The immunofluorescence staining intensity was graded according to the Mayo Clinic/Renal Pathology Society Consensus Guidelines(10) using a semi-quantitative scale: “negative, ±, 1+, 2+, and 3+”.

RESULTS: -

Eighty-nine renal biopsy samples were received at the Department of Immunopathology during a one-year research span. Out of these, four biopsies lacked sufficient clinical information for evaluation. In twenty cases, the biopsy material was deemed inadequate for routine light microscopy and/or direct immunofluorescence (DIF) analysis. Specifically, nine of these biopsies were non-representative, while the remaining eleven contained only medullary tissue with no identifiable glomeruli. Consequently, comprehensive histopathological and immunopathological evaluation was performed on the remaining 65 adequately sampled renal biopsies.

Patient Characteristics:-

The study samples consisted of 73% (65/89) adequately sampled renal biopsies. The majority of the patients, 36.9% (24/65) were within 21 - 30 years old. The ages of the study participants were

between 11 and 70 years, with a mean ± SD of 30.81 ± 5.02 years. Of the study participants, 38 (58.5%) were male, resulting in a male to female ratio of 1.4:1, as illustrated in table 1.

Table 1 - Allotment of renal disease with gender distribution

Renal Disease	No of cases n (%)	Males n (%)	Females n (%)
Focal segmental glomerulosclerosis	18 (27.7)	11 (61.1)	7 (38.9)
Membranous glomerulonephritis	11 (16.9)	6 (54.5)	5 (45.5)
Minimal change glomerulopathy	9 (13.8)	6 (66.7)	3 (33.3)
Membranoproliferative glomerulonephritis	6 (9.2)	2 (33.3)	4 (66.7)
IgA nephropathy	5 (7.7)	4 (80.0)	1 (20.0)
Rapidly Progressive glomerulonephritis	4 (6.2)	2 (50.0)	2 (50.0)
Lupus nephritis	4 (6.2)	1 (25.0)	3 (75.0)
Diabetes nephropathy	3 (4.6)	2 (66.7)	1 (33.3)
Acute tubulointerstitial nephritis	2 (3.1)	2 (100)	–
Mesangioproliferative glomerulonephritis	2 (3.1)	1 (50.0)	1 (50.0)
Hemolytic uremic syndrome	1 (1.5)	1 (100)	–
Total	65 (100)	38 (58.5)	27 (41.5)

Clinical manifestations in Renal Disease:-

The primary reason for performing a renal biopsy was nephrotic syndrome, with haematuria being the second most common. Table 2 displays the clinical manifestations.

Focal segmental glomerulosclerosis supposed for 27.7% (18/65) of total renal biopsy diagnoses, succeeded by membranous glomerulonephritis 16.9% (11/65).

Table 2 - Clinical manifestations in diverse kidney ailments

Renal Disease (n)	Clinical Presentation n (%)					
	Nephrotic Syndrome	Acute Nephritis	ARF	CRF	RPRF	Haematuria
Focal segmental glomerulosclerosis (18)	11 (61.1)	–	3 (16.7)	3 (16.7)	–	1 (5.5)
Membranous glomerulonephritis (11)	8 (72.7)	–	–	2 (18.2)	–	1 (9.1)
Minimal change glomerulopathy (9)	9 (100)	–	–	–	–	–
Membranoproliferative glomerulonephritis (6)	4 (66.6)	–	1 (16.7)	–	–	1 (16.7)
IgA nephropathy (5)	2 (40)	–	–	–	–	3 (60)
Rapidly Progressive glomerulonephritis (4)	–	3 (75)	–	–	–	1 (25)
Lupus nephritis (4)	2 (50)	–	–	–	1 (25)	1 (25)
Diabetes nephropathy (3)	3 (100)	–	–	–	–	–
Acute tubulointerstitial nephritis (2)	–	1 (50)	–	–	1 (50)	–
Mesangioproliferative glomerulonephritis (2)	1 (50)	–	–	–	–	1 (50)
Hemolytic uremic syndrome (1)	–	–	–	–	1 (100)	–

ARF: Acute Renal Failure, CRF: Chronic Renal Failure, RPRF: Rapidly Progressive Renal Failure

DIF findings in Renal Specimens:-

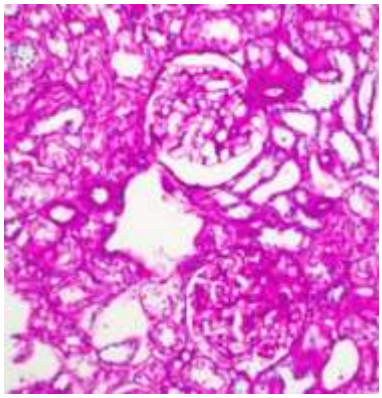
Direct immunofluorescence (DIF) was performed on all 65 cases. Among these, 38 cases (58.5%) demonstrated positive immunofluorescence findings, while 27 cases (41.5%) showed no detectable immune deposits. The 27 negative cases -- 12 cases of acute tubulointerstitial nephritis, 8 of minimal change glomerulopathy, 4 of diabetic nephropathy, 2 of hemolytic uremic syndrome, and 1 case of membranoproliferative glomerulonephritis.

The most frequent type of single immune deposits in various combinations was C3 [94.7%, (36/38)] followed by IgG [68.4%, (26/38)] and IgA [47.4%, (18/38)]. The most common combination of deposits was IgG with C3, observed in 11 cases (28.9%), followed by IgA with C3, detected in 8 cases (21.0%), as depicted in table 3.

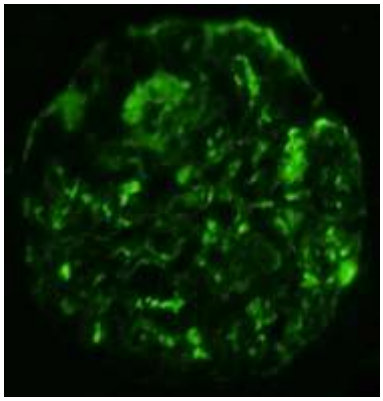
Table 3- Frequency of immunoreactive positivity in DIF positive cases

Immunoreactive Positivity	Number of cases n (%)
ImmunoglobulinG + C3	11 (28.9)
ImmunoglobulinA + C3	8 (21.0)
ImmunoglobulinA + ImmunoglobulinG + C3	6 (15.8)
ImmunoglobulinM + ImmunoglobulinG + C3	5 (13.2)
ImmunoglobulinM + ImmunoglobulinG + ImmunoglobulinA + C3	4 (10.5)
ImmunoglobulinM + C3	2 (5.3)
C3 alone	2 (5.3)
Total	38 (100)

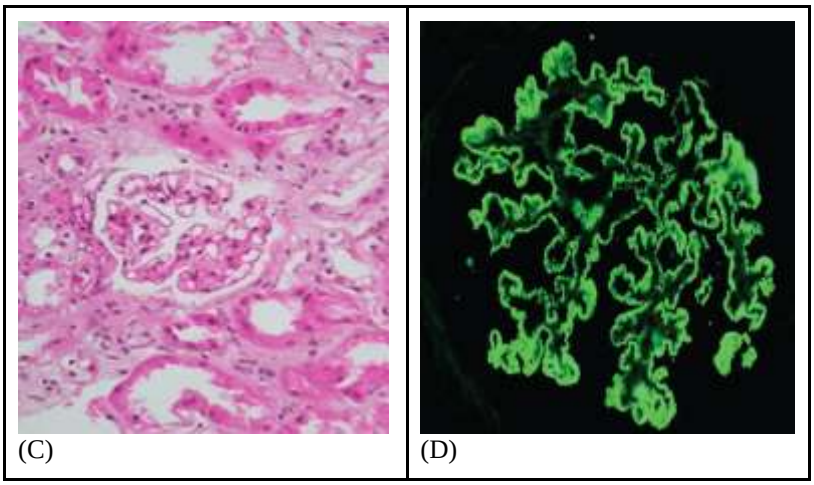
Light microscopy and Direct Immunofluorescence of different kinds of GN are illustrated in Fig 1-3.



(A)

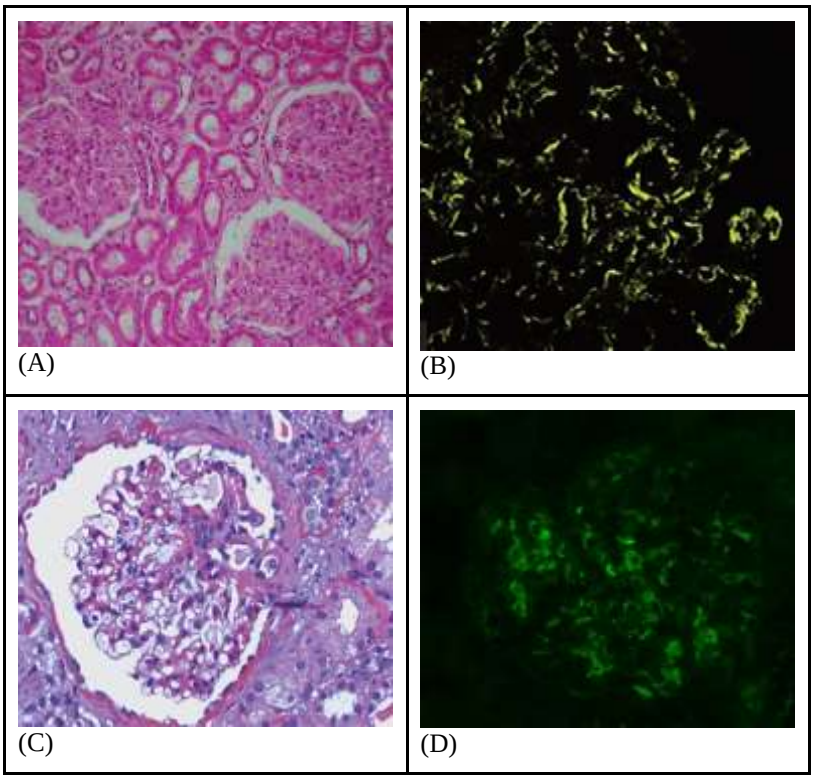


(B)



(C) (D)

Fig 1 - (A) Segmental glomerular sclerosis in focal segmental glomerulosclerosis (FSGS) (PAS, ×40) (B) DIF of FSGS revealing IgM (2+) deposits in sclerotic areas (×400) (C) Membranous glomerulonephritis exhibiting thickening of the GBM; with the capillary loops appear stiff and rounded (H and E, ×400) (D) Direct immunofluorescence (DIF) of membranous GN reveals granular IgG (2+) deposits along the capillary walls (×400)



(A) (B)

(C) (D)

Fig 2 - (A) Membranoproliferative glomerulonephritis (MPGN) exhibiting mesangial matrix enlargement combined with lobular accentuation (H and E, ×400) (B) DIF indicates C3 (2+) along the walls of capillaries in MPGN (×400) (C) IgA nephropathy exhibiting mesangial proliferation/expansion (PAS, ×40) (D) DIF demonstrating mesangial deposits of IgA (2+) in IgA nephropathy (×400)

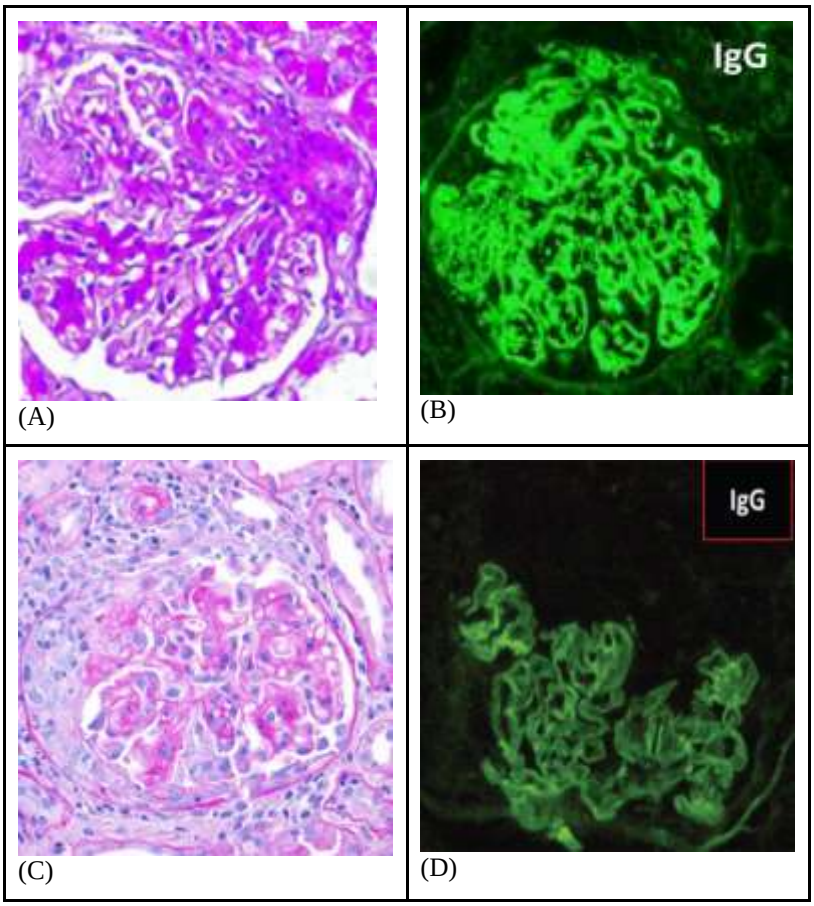


Fig 3 - (A) Lupus nephritis demonstrating a glomerular tuft featuring mesangial cell proliferation, areas with endocapillary proliferation, and a small well-defined crescent (PAS, ×40) (B) Lupus nephritis DIF reveals IgG immune deposits in the glomerular capillary wall in a focal pattern, along with mesangial deposits present (×400) (C) RPGN DIF displaying interstitial inflammation, cellular crescents, and slight mesangial matrix expansion (PAS, ×40) (D) RPGN DIF displaying linear IgG deposits along the GBM (×400)

DISCUSSION: -

Glomerulonephritis refers to a broad spectrum of kidney diseases marked by inflammation of the glomeruli, which can result in substantial deterioration of renal function(11). Precise diagnosis and proper classification are essential for determining appropriate treatment strategies and assessing disease prognosis. Histopathological examination, particularly via light microscopy, plays a foundational role in identifying structural changes such as hypercellularity, basement membrane thickening, and crescent formation(12). However, light microscopy alone may not sufficiently distinguish between the various immune-mediated etiologies. In this context, direct immunofluorescence (DIF) emerges as an indispensable diagnostic tool, enabling the visualization of immune complex deposits and complement components with high specificity. By detecting the site, vehemence, and constellation of Ig and complement deposition, DIF enhances diagnostic precision, particularly in differentiating between diseases with overlapping histologic features on light microscopy(13).

In our study, renal disease was epidemiologically substantially higher in males (58.5%) than females (41.5%), with male preponderance of 1.4:1, excepting lupus nephritis, comparable to findings by Li et al(14) and Jalalah(15). The highest prevalence of glomerulonephritis in the age bracket of 21–30 years, parallel with observations made by Hossain et al(16), Minz et al(17) and Salahuddin et al(18), whereas in disagreement with Wetmore et al(19).

In the present research, morphologically, focal segmental glomerulosclerosis (FSGS) was the most frequent cause of GN (27.7%). Concordant results exist in earlier research(20–22). These results are in disagreement with Yim et al(23) and Ellison et al(24).

Nephrotic syndrome (61.3%) was the prevalent clinical manifestation among the study population, which aligns with the work of Paksoy et (25) and Wendt et al(26).

In current research, the prevalent immune deposit on IF was observed to be C3, accounting for 94.7%. The results are analogous to other similar studies(27,28).

CONCLUSION: -

Direct Immunofluorescence remains a cornerstone in the diagnostic armamentarium for renal diseases. Its ability to reveal the immunopathological landscape of glomerular disorders provides invaluable insights that enhance diagnostic accuracy, guide therapeutic decisions, and ultimately improve patient outcomes. Continued emphasis on Direct Immunofluorescence represents the optimal strategy for comprehensive renal biopsy interpretation in clinical practice.

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